THE NEW YORK ACADEMY OF SCIENCES

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The Sections and the Division hold meetings regularly one evening each month during the academic year October to May inclusive All meetings are held at the building of The New York Academy of Sciences, 2 East Sixty third Street New York 21 New York

Conferences are also held at irregular intervals at times announced by special programs

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ANIMAL DISEASE AND HUMAN HEALTH*

Conference Chairman and Consulting Editor
JAMES LIEBERMAN

CONTENTS

* This series of papers is the result of a conference on *Animal Disease and Human Health* held by The New York Academy of Sciences in collaboration with the Communicable Disease Center, Public Health Service, Atlanta, Ga., September 11, 12, and 13, 1957.

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Part III Leptospirosis: A Contemporary Problem

Part IV Recent Developments in the Epidemiology of Rabies

Part V Ornithosis

Part VI Other Contemporary Disease Problems

Part VII Selected Chronic Diseases

Part VIII International Programs

Summary

FOREWORD

By James Lieberman

Communicable Disease Center Public Health Service Atlanta Ga

This monograph comprises a publication on comparative medicine that should prove useful to the professions of the medical sciences

An editorial that appeared in *The New York Times* (September 12 1957) said in part of the conference on which this monograph is based The relationship of animal disease to human welfare although recognized through the ages has not been explored as thoroughly as it might be There is a tendency to forget that the dawn of bacteriology emerged through the pioneering efforts of such men as Louis Pasteur with his work on anthrax a disease affecting lower animals as well as man

The conference is a ten year progress report on the zoonoses' the category of diseases of lower animals transmissible to man Diseases such as psittacosis brucellosis cat scratch fever toxoplasmosis and a host of others will be reviewed with accompanying progress reports on methods for their diagnosis and control Other diseases not included in this category will be discussed from the standpoint of their possible relationship to similar conditions in man For example pulmonary adenomatosis which has been decimating sheep flocks in many areas of the world has several factors in common with lung cancer in humans

Although the subject of this monograph can hardly be dealt with adequately in a publication of this size perhaps it will be agreed that the subject matter selected illustrates the inseparability of those diseases that affect both man and the lower animals The contributors to these pages have not been concerned solely with the acute communicable diseases but have ventured also into the comparative pathology of several of the chronic diseases and the chronic sequelae to infectious diseases There are special papers dealing with global activities in this field and with biological warfare In each instance topics are discussed by persons well versed in their respective specialties Limitations of space alone have prevented amplifying the scope of this publication

In a very broad sense comparative medicine and its subspecialties have been commented upon since the Biblical era Medical and veterinary texts have devoted much time to this subject but one of the first sessions devoted to *The Relation of Diseases in the Lower Animals to Human Welfare* was sponsored by The New York Academy of Sciences in 1946 these proceedings were published by the Academy in April 1947 It is expected that this volume will supplement rather than completely supplant the earlier publication and that it will clarify and bring up to date many concepts understood imperfectly or not at all ten years ago

Introduction

ANIMAL DISEASES THEIR RELATIONSHIP TO THE HEALTH OF MAN

By J D Martin*

Section of Epidemiology Louisiana State Department of Health New Orleans La

Significance of the Relationship of Animal Health to Human Health

The health of humans is inextricably related to the health of animals Recognition of this fact dates to antiquity but the closeness and full significance of the relationship is just beginning to be seen in its proper perspective

Diseases, both human and animal gravely influence man s affairs National *and international and also personal health and welfare are dependent* to a large extent upon the prevention of disease The need of the United States to be prepared for any eventuality poses a problem for today and tomorrow and such preparedness requires healthy citizens This is secured by a healthful economy and in addition to other public health measures an adequate supply of safe wholesome food to meet the world s peacetime and wartime needs as well as an adequate system for its distribution The achievement of this state of preparedness possible only when men and animals are healthy will be attained only when people become aware of the problems confronting them and when they apply all available knowledge and skills to resolve these problems

More than 200 diseases of animals are communicable almost half are known to be transmissible from animal to man There are also more than 100 different parasites that affect both man and animals

As investigative methods improve the number of disease entities due to hitherto unidentified infectious and noninfectious processes will increase steadily and will continue to increase as man applies himself to a study of his nature and his environment

In addition to the direct effect of animal diseases on human health through the impact of infectious diseases transmissible to man human health and well being are also significantly influenced by animals through the food and raw materials derived from them through recreation and sports associated with them and through the opportunities they offer for research into basic problems affecting man Animals serve man as food pets beasts of burden and means of transportation At times they also affect him as enemies intent upon his injury or destruction

The interrelationship between animals and man is intimate significant and far reaching If man is to continue to improve his health he must recognize the relationship study it carefully and apply the knowledge gained

Historical Recognition of the Relationship

Man s knowledge of this relationship of animal disease to human health dates from antiquity Control measures were developed even before the rec

*Present address State of Louisiana Department of Public Welfare Baton Rouge La

ognition of the infectious nature of some of the diseases of animals transmissible to man

The Bible tells us that Abel the son of the first man and woman offered of his flocks to the Lord This account, with that of Adam and Eve in the Garden of Paradise indicates that the relationship of animals to man was recognized from the earliest days of human existence

Study of the history of diseases and their manifestations strongly suggests that indubitable evidences of human disease[1] appeared almost synchronously with the first manifestations of human life on the earth

Mettler expresses doubt that particular etiologic agents of specific diseases have ever been completely absent He quotes Moodie[2] as saying that parasitism is a most antiquated mode of life and has been proved to extend as far back as the paleozoic period

In Biblical times the capture of the Ark of the Covenant by the Philistines was associated with an epidemic probably bubonic plague that killed 50 000 Bethshemites alone That true bubonic plague was recognized from very ancient times appears probable as the association of rats with disease of bubonic nature is also indicated in the cuneiform writings of Babylonia [3]

A celebrated passage in Susruta[4] is interpreted as indicating that early Indian medicine had knowledge of the relationship between mosquitoes and malarial fever A warning to abandon a dwelling when its rats act queerly and die also suggests to Castiglioni[1] that the ancient Persians and Indians suspected the relationship between plague and rats

Bubonic plague and louse borne typhus probably were known to the Romans also History suggests frequent epidemics of bubonic plague with thousands of persons dying and Castiglioni tells of the pestilence called the Plague of Antoninus or of Galen that lasted from 164 to 180 A D and was probably louse borne typhus although it could have been bubonic plague Historically the diagnosis is still uncertain

There is evidence too that throughout the classical Greek period numerous endemic foci of plague and malaria existed [3]

We also find in the Bible an account of a disease that in the time of Moses destroyed all the beasts horses asses oxen camels and sheep This disease could have been anthrax

Rabies was one of the first recognized zoonoses Kelser[5] quotes Aristotle as writing in the Fourth Century B C that dogs suffer from madness which puts them in a state of fury and all animals which they bite when in this condition become also attacked with madness

Malaria too has been known from ancient times Frightful epidemics of malaria wreaked havoc in Rome in the later periods of the Roman Empire and the people associated malaria with swamps and drained them in an effort to control it

Tsutsugamushi fever or mite typhus is believed by Williams[6] to have been recognized as an entity since the Sixth Century A D Blake and his co workers[7] believe that it afflicted the natives of south China in the Sixteenth Century

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Epidemics of louse borne typhus have probably occurred everywhere and in

every period of history where people have lived under crowded unsanitary conditions

As urbanization developed in the medieval period the city, which was such an important factor in economic prosperity became an equally important factor in the spread of disease The crowding of people into cities and the almost constant military conflict between these cities with the resultant movement of armies spread disease everywhere The most serious of the diseases thus spread was bubonic plague Zinsser[8] quotes J F C Hecker s estimate that at least 25 million people died in Europe of this disease during this period

The development of cities also resulted in the adoption of laws to protect the health of mankind During the Dark Ages history is silent about sanitary regulations concerning animals and animal products until 1350 A D when John II of France established sanitary police as a governmental instrument[9] Two of the functions of this regulatory force were to keep hogs out of cities and to prevent butchers and fishmongers from selling spoiled meat and fish After 1350 A D step by step the sanitary regulations that presently protect man were developed

Impact of Animal Diseases on Man

Animal diseases directly and indirectly affect man s physical mental emotional social economic and political well being The direct impact is evidenced by the morbidity and mortality resulting from animal pathogens that infect man The indirect results are shown by the effect of animal diseases on man s economy his recreational activities and his social and political aspirations

To estimate the number of persons who annually become ill or die as a result of infections transmitted by animals is well nigh impossible Even in the United States many of these conditions affecting man go unrecognized and unreported In less fortunate countries where these diseases are more common reporting is usually even less reliable so that attempted estimates of morbidity or mortality would probably be greatly in error on the conservative side

As one studies the staggering total of deaths and hours of absenteeism due to zoonoses by comparison the deaths and disabilities resulting from wars appear negligible Accurate statistics are not available but it is estimated that 21 million persons died of influenza in the pandemic of 1918 and 1919 while 200 million more were made ill Whether or not this pandemic can be traced to animals we shall probably never know but some persons believe that the infectious agent that caused it was the swine influenza virus During and following the Balkan wars and World War I typhus fever appeared in epidemic form in all the countries of eastern and central Europe It is estimated that at least 1 million persons died in these countries during and following this world war[10]

In our own country yellow fever made its appearance in the late Seventeenth Century In 1907 Carroll[11] estimated that yellow fever had caused 100 000 deaths in the United States since 1793

The epidemics that decimated the Indian peoples of Mexico and helped to

destroy their civilization could have been due, to a large extent, to yellow fever, bubonic plague, or typhus fever. According to the writings of Padre Sahagun,[1] a great epidemic of typhus carried off about 2 million Indians in Mexico in 1577 A.D.

Plague, a disease of antiquity that has caused millions of human deaths in the years during which it has been prevalent, is also a disease of the present. One of the latest epidemics of great magnitude occurred in Manchuria in 1910–1911, killing more than 50,000 persons,[12] but bubonic plague smolders in many endemic foci throughout the world and, if the right conditions develop, it can again become epidemic or pandemic.

Animal diseases transmissible to man are still with us. Some of these are capable of causing large numbers of human deaths, inestimable human suffering, dislocation of large numbers of people, and upsets in emotional, social, and economic life, and of serving as a contributing factor in changes in political power.

Animals benefit man through various uses. Anything, therefore, that improves the health status of animals contributes clearly to the health and well-being of man.

Losses in animals affect humans in several ways: by the increased cost of production of domestic animals, by the reduced quantity and quality of animal products available to the consumer, and through diminished opportunity for recreation.

In addition to losses due to the death of animals, diseases in animals also cause losses in milk, in meat and egg production, in hides because of poor quality, in work days for dray animals, in wasted feed and labor for animal care, and in loss of offspring in some infectious and reproductive diseases.[14] Moreover, the livestock owner undergoes considerable inconvenience and the additional cost necessary to replace the sick or dead animal.

This inconvenience and cost is not borne by the livestock owner alone, however; it is also borne by every man, woman, and child everywhere in increased taxes and increased costs of food, food products, and the necessities of life derived from animals.

The United States Department of Agriculture estimates the annual livestock loss from all causes in this country at more than 2 billion dollars a year.[14] About one half of this loss is due to parasites. Animal death losses from all causes in the United States in 1954 included more than 1.5 million cattle, 2.5 million calves, 4 million sheep and lambs, 10.5 million hogs and pigs, 235 million chickens, 7.2 million turkeys, and about 250 thousand horses and mules, together with an unknown number of goats, fur-bearing animals, domestic rabbits, and miscellaneous poultry.[15] Losses in the United States each year are staggering and, in many instances, unnecessary. In addition to losses on the farm there are others not counted in the preceding statement, such as those resulting during transportation from the farm to the market, in processing and packaging plants, and from the condemnation of carcasses, hides, edible offal, and other by-products, in whole or part, because of disease or parasites. If we add to the costs of the obvious and hidden losses the cost of controlling or preventing diseases and parasites and the cost of protecting the public from un-

wholesome meat and meat products the total may well approximate 5 billion dollars a year and this in a country such as the United States What then must be the cost of livestock losses to so many of the nations of the world that are not as fortunate? The impact of animal disease on man s economic status is terrific

Briefly too it is necessary to note the impact of animal diseases on man s recreational activities Epizootics kill off many of the animals that man hunts for pleasure and so decrease his opportunity to pursue this sport Certain of these diseases in game animals also endanger human life

Zoonoses also prevent the full use of several areas in the world that are otherwise well suited to the production of livestock To feed the world s growing population it is necessary that every acre of land available for livestock production be utilized Unfortunately much of the land in question is in poor and technically undeveloped countries Rinderpest prevents the development of the potentially great grazing areas of Africa Perhaps the most important of the zoonoses of wild animals at this time is African trypanosomiasis which affects man not only directly in its impact on his health but because of its economic effect Because of this disease an area of grazing land in central Africa approximately 4 million square miles in extent remains economically poor[16] This area would be wonderful range country for livestock if trypanosomiasis could be eradicated or controlled Control of African swine disease in coastal Africa would permit hog production in Africa to become a reality and this immense continent could then contribute great quantities of meat to a world starving for animal protein[17] The problem is not confined to Africa however if mastitis could be controlled and prevented in the dairy herds of Europe and in the United States milk production could be increased appreciably This is important as there is a direct relationship between longevity and per capita income and the percentage of animal protein in the diet (TABLE 1)[18]

We who live in the United States are singularly blessed There are few other places where food supplies can be taken for granted Perhaps one half of the world s people exist on diets that are nutritionally poor[17] Protein malnutrition is found wherever animal diseases and parasites are uncontrolled Clarkson[17] says that the average person in the world usually has less meat in a year than most citizens of the United States eat in a week This is to a significant degree the result of uncontrolled parasitism and animal disease

Extent of the Problem

The problem is steadily increasing in magnitude About one half of the people of the world are on deficient diets These people need animal proteins yet unless a concerted effort is made to bring all of the world s technical knowledge skills and financial resources into play to control animal diseases and parasites the present problem will be even greater in the future

To supply an average family of four in the United States three tons of food a year are required[17] More than one half of this is meat eggs poultry and dairy products in 1956 25 per cent more of these animal foods were consumed there than in 1935 The United States has only a small percentage of the world s peoples but its farms produce one third of the world s recorded yearly

total of 80 billion pounds of red meat and one fourth of the 500 billion pounds of milk[17]

The world s population is increasing steadily These people must be fed adequately To produce the necessary food resources the world over must be utilized to the fullest for the production of livestock To accomplish this parasites pestiferous insects and vectors of disease must be controlled and eradicated Such an achievement would contribute greatly to better health and improved standards of living for all the people of the world

All animals domestic and wild are infested with parasites that contribute to man s burden of disease In some areas parasitic infestation is so great that man s struggle against these organisms is part of his struggle to survive Control of parasites therefore should be one of man s goals Fortunately it is an attainable one

The fight against animal disease is international in scope Sometimes the battle against disease must be fought by a nation outside its national boundaries An example of this is the successful campaign that the United States has just waged in Mexico at a cost of more than 134 million dollars to eradicate foot and mouth disease[15] The Mexican government spent a similar large sum of money in this fight[16] but foot and mouth disease is now under control in that country The vast meat resources of Uruguay and Argentina are also barred from the United States because of the danger of importing the same disease[16]

The problem of animal disease and human health has another very serious implication Today in this world of stress where nations align themselves against one another the threat of biological warfare is no longer just a theory but a real possibility and a number of the agents that cause animal disease are adaptable to biological warfare[18] It behooves us therefore to become increasingly aware of this danger to become familiar with the clinical picture of these diseases in animals and man to be ready to apply necessary control measures and in the meantime to work to develop faster and more accurate ways of identifying agents of disease and of producing and using immunizing agents

Research

The advancement of medical knowledge has been and is inseparably linked with animal experimentation Experiments on animals have led and in the future may be expected to lead to great benefits to man and to animals Man s contest with external nature is expressed in two different ways adaptation to or changing of a given environment[19] Research assists in this struggle

Through the centuries man has been concerned with the development of knowledge to cure diseases Today science is more concerned with the development of methods of preventing disease by eliminating its cause

Great promise appears in the use of genetics as a tool for eliminating pathological abnormalities and disease Studies with animals have shown that certain characteristics can be bred in or out of animals and that certain animals have a genetic or natural resistance to specific disease[20] Such studies must be continued

Research into the effect of environmental factors is important also in the

TABLE 1

LONGEVITY AND ECONOMIC DEVELOPMENT*

	Expectation of life at birth (1)		National income per capita 1939 (3)	Food: available calories per person per day (2)	Available total protein per person per day (2)	Available animal protein per person per day (2)	Total protein that is animal	Infant mortality 1936–1940	Death rate 1936–1938 (4)	Birth rate 1936–1938 (4)	Illiterate population 10 years and over (5)		Urban population (5)	Population type (3a)
	Years	Period	Dollars		Grams	Grams	Per cent				Per cent			
India	26.7	1931	34	2021	56	9	16	166.0	23.7	34.9	91(10)	(1931)	13	3
Egypt	30.9	1927–37	85	2199	69	8	12	205	30.0	45.8	85(11)	(1937)	25	3
China	34.7(6)	1929–31	29	2201	68	5	7			45.0†				3
Mexico	37.2	1929–33	61	1909	59	20	34	125.5	26.1(9)	43.5	52	(1940)	35	3
Korea	37.5	1931–35		1904	70	15	21				69	(1930)	7	
Peru	39.0(7)	1933–35	72	2090	58	8	14	128.0	15(9)	32.0	57(12)	(1940)	35	3
Chile	41.2	1939	174	2481	70	24	34	236	24.4	33.5	28	(1940)	52	
Brazil	43.0	1939–40	46	2552	73	26	36				57	(1940)	31	3
Colombia	46.3	1939–41	76	1934	62	29	47	158.0	15.5	32.1†	44	(1938)	22	3
Bulgaria	46.3	1925–28	109	2831	90	18	20	149	15.6	29.5	31	(1934)	20	2
Japan	48.3	1935–36	93	2268	67	12	18	112.2	17.7(9)	29.4†	10	(1935)	47	2
Poland	49.8	1931–32	95	2702	72	19	26		14.4	26.8	23	(1931)	30	2
Hungary	49.8	1930–31	125	2815	69	24	24	147.3	15.3	22.0	6	(1941)	36	1
Greece	[illegible]0.0	1928	136	2523	65	14	22	115.0	16.3	29.3	41	(1928)	35	2
U.S.S.R.	50.0(8)	1926–27	158	2815	71	17	24		17.8(9)	40.0				2
Portugal	[illegible]0.7	1939–42		2461	74	23	31	144	17.0	29.0	49	(1940)	31	
Czechoslovakia	53.6	1929–32	134	2761	72	25	35		13.5	18.4	4	(1930)	48	1
Italy	54.9	1930–32	140	2627	81	19	23	102.4	14.1	23.8	22	(1931)	44	1
Austria	56.5	1930–33	166	2933	79	36	46	99	13.6	14.3		(1935)	63	1
Finland	57.0	1931–40	184	2950	80	37	46	73	13.6	19.9	1(13)	(1930)	24	1
Ireland	57.1	1925–27	248	3184	92	41	45	66.3	14.2	19.5		(1946)	38	1
Belgium	57.9	1928–32	261	2885	77	32	42	[illegible]6.7	13.3	16.5	6(14)	(1930)	61	1
France	58.8	1933–38	283	3012	87	38	44	65.8	15.7	16.2	4	(1936)	53	1
Germany	61.3	1932–34	520	2967	77	34	44	61.0	11.6	18.0	‡	(1936)	66	1
United Kingdom	61.5	1937	468	3005	80	43	54	59.5	12.0	15.1	‡	(1931)	[illegible]9	1
Norway	62.4	1921–31	279	3129	83	41	49	38.8	10.4	15.6	0	(1935)	27	1
Switzerland	62.9	1931–41	445	3049	89	48	54	43.9	11.6	16.4	‡	(1941)	33	1

Canada	64 6	1940–42	387	3109	87	47	54	60 1	9 9	20 6	4	(1931)	54	‡
Denmark	64 7	1936–40	338	3249	6	44	58	55 6	11 0	18 0	0	(1935)	63	‡
United States	65 0	1939–41	554	3249	58	50	57	48 7	11 1	17 4	4	(1930)	56	‡
Australia	6 3	1932 34	403	3128	90	59	66	38 3	9 4	17 4	0	(1930)	64	‡
Sweden	65 6	1936–40	436	3052	68	54	61	40 4	11 7	14 5	0	(1930)	42	‡
Netherland	66 5	1931–40	338	2958	8	37	47	36 2	8 8	20 8	0	(1935)	52	‡
New Zealand	66 9	1934–38	336	3281	36	61	64	32 2	8 5	17 3	0	(1935)	60	‡

(1) Most of the data are from L I Dublin, A J Lotka and M Spiegelman *Length of Life* revised 1947 Ronald Press New York N Y
(2) *World Food Survey* July 1946 FAO United Nations
(3) H S Lique t 19 0 World Labour and World Production *Annals of the American Academy of Political and Social Sciences* v 1 268 p 148
3a) (From Liquet *ibid*) Population types are as follows Type 1—*Low growth potential* Birth rates below 25/1000 pop Low death rates Small natural increase with prospect of relatively stationary population in the future Type 2—*Transitional growth* Birth rates approximately between 25 and 35 Both birth and death rate generally falling Rapid population growth Exception is U S S R high birth rate Type 3—*High growth potential* Birth rates around 35 or over Death rates (but not birth rates) generally declining Rapid growth in absence of civil disturbance famine and epidemic
(4) *Epidemiological and Vital Statistic Reports* from World Health Organization
(5) *Statistical Yearbook* and *Demographic Yearbook* 1948 United Nations Dates in column on illiteracy apply to column on urbanization
(6) Rural China
(7) For the city of Lima
(8) White Russia and the Ukraine
(9) Data for 1932
(10) Excluding 17 million people in outlying area
(11) Excluding nomadic population
(12) Not including 350 000 jungle population and 46 144 underenumeration of population
(13) Fifteen years and over
(14) Seven years and over
Reproduced (slightly adapted) by permission from *The Journal of the Louisiana State Medical Society* formerly the *New Orleans Medical and Surgical Journal*[11]
† The birth rate for China is an approximation for Colombia and Japan 1938 1944
‡ Less than 5 per cent illiteracy

TABLE 1
LONGEVITY AND ECONOMIC DEVELOPMENT

	Expectation of life at birth (1)		National income per capita, 1939 (3)	Food available per person per day (2)	Animal protein per person per day (2)	Animal protein per person per day (2)	Total protein that is animal	Infant mortality 1936–1940	Death rate 1936–1938 (4)	Birth rate 1936–1938 (4)	Illiteracy over 10 years (5)		Urban population (5)	Economic type (6)
	Years	Period	Dollars	Calories	Grams	Grams	Per cent				Per cent	Year		
India	26.7	1931	34	2021	56	9	16	166.0	23.7	34.9	91(10)	(1931)	13	3
Egypt	30.9	1927–37	55	2199	69	8	12	205	30.0	45.8	85(11)	(1937)	25	3
China	34.7(6)	1929–31	23	2201	68	5	7			45.0†				3
Mexico	37.2	1930–33	61	1909	59	20	34	[illegible]	26.1(9)	43.5	57	(1940)	35	3
Korea	37.5	1931–35		1904	60	15	21				69	(1930)	–	
Peru	39.0(7)	1933–35	72	2090	58	9	14	128.0	15(9)	32.0	57(12)	(1940)	35	3
Chile	41.2	1939	174	2481	60	24	34	236	24.4	33.5	28	(1940)	52	
Brazil	43.0	1939–40	46	2552	73	26	36				57	(1940)	31	3
Colombia	46.3	1939–41	[illegible]	1934	62	29	41	158.0	15.5	32.1†	44	(1938)	29	3
Bulgaria	46.3	1925–28	109	2831	90	18	0	149	15.6	29.5	31	(1934)	20	2
Japan	48.3	1935–36	93	2268	[illegible]	12	18	117.2	17.4(9)	23.4†	10	(1935)	47	2
Poland	49.8	1931–32	9	[illegible]	2	19	26		14.4	6.8	23	(1931)	30	2
Hungary	49.8	1930–31	125	2815	59	24	27	147.3	15.3	27.0	6	(1941)	36	1
Greece	50.0	1928	136	2573	65	14	22	115.0	16.3	29.3	41	(1928)	35	2
U S S R	50.0(8)	1926–27	158	2815	71	17	24		17.8(9)	40.0				2
Portugal	50.7	1939–42		2461	74	23	31	144	17.0	27.0	49	(1940)	31	
Czechoslovakia	53.6	1929–32	134	2761	72	25	35		13.5	18.4	4	(1930)	48	1
Italy	54.9	1930–32	140	2627	81	19	23	102.4	14.1	23.6	22	(1931)	44	1
Austria	56.5	1930–33	166	2933	9	36	46	99	13.6	14.3		(1935)	63	1
Finland	57.0	1931–40	184	2950	60	37	46	73	13.6	19.9	1(13)	(1930)	24	1
Ireland	57.1	1925–27	248	3184	92	41	45	66.3	14.2	19.5		(1946)	38	1
Belgium	57.[illegible]	1928–32	261	2885	67	32	42	[illegible]	13.3	16.5	6(14)	(1930)	61	1
France	58.8	1933–38	283	3012	87	38	44	65.8	15	16	4	(1936)	53	1
Germany	61.3	1932–34	520	2967	77	34	44	61.0	11.6	18.0	‡	(1936)	66	1
United Kingdom	61.5	1937	468	3005	80	43	54	53.5	12.0	15.1	‡	(1931)	[illegible]	1
Norway	62.4	1921–31	[illegible]	3129	83	41	49	38.8	10.4	15.6	0	(1935)	27	1
Switzerland	67.9	1931–41	445	[illegible]	[illegible]	48	54	43.[illegible]	11.6	16.4	‡	(1941)	33	1

many of these pests Man aggravates the problem by himself becoming the transporter of destructive pests and pathogens from farm to farm state to state nation to nation and continent to continent

Changing environmental conditions also favor the establishment of new pests and insect vectors of disease

The movement of livestock to auction barns and agricultural fairs has augmented the spread of insect pests favored the transmission of communicable diseases and increased the spread of parasites

Rodents and other wild animals and birds periodically migrate in large numbers and carry dangerous insect life and parasites with them Wherever trade moves rats follow and with the rats the diseases commonly associated with them In addition many other wild animals (for example the hamster) and wild birds are moving in commercial channels to be sold as pets Each of these is a potential source of human disease

Van Houweling[3] tells an interesting story that highlights the increasing problem caused by today s transportation systems in the spread of disease In 1843 a New York milkman Peter Dunn bought a milk cow from the captain of the *Washington* a British ship It seemed to Dunn that he was getting the cow at a big bargain As it turned out however this cow was just as expensive as the famous cow of Mrs O Leary which supposedly started the fire that destroyed one third of Chicago in 1871 Dunn s cow introduced contagious pleuropneumonia into the United States and cost our livestock owners a fortune before the disease was brought under control and eradicated In addition this program cost the national government 5 years of work and $1 509 100 72 before it was completed Pertinent to my point however is the fact that pleuropneumonia in spite of its contagiousness spread slowly because at that time transportation was slow and the movement of livestock was limited Similarly hog cholera required 15 years to spread across the land after its identification in 1833 and tick fever moved across North Carolina at a rate of only 4 miles a year in the 1870s[17] Contrast these spreads with the speed with which starting in California in 1952 a vesicular exanthema of hogs swept through 20 states in 3 months[17] and the problem of infectious disease control today becomes obvious

This does not mean that livestock should not be moved from place to place but it does suggest that only healthy livestock should be moved and then only under sanitary conditions

Relationship to Human Medicine

The influence that animal health exerts on human health and welfare is real There is a grave need for physicians and other scientists to become much more appreciative of this influence so that they may be prepared to guide people into an awareness of the problem so that through their cooperative efforts necessary control measures may be instituted

As mentioned earlier there is also a need for physicians to familiarize themselves with the human manifestations of the zoonoses and the best methods of treating these diseases

Research people and epidemiologists must assist physicians with studies of

study of health and disease Because of their relatively short lives and their expendability animals lend themselves readily to use for research involving generations or serial studies

Many of the diseases to which man is heir such as abnormalities of growth and development metabolic and nutritional disorders poisonings and infections also occur in animals Research into the nature and control of animal diseases can precede and be a pattern for similar studies in related human diseases

As far back as 40 B C Celsus[1] foresaw that clinical work with animals would later find a place in the practice of human medicine This observation was emphasized by Galen 200 years later but progress along remedial lines was slow and halting for centuries and at the end of the Eighteenth Century in spite of early descriptions of animal plagues knowledge of animal diseases was still in the folklore stages[1] Louis Pasteur Robert Koch Theobald Smith Sir Alexander Fleming and others in the late Nineteenth and early Twentieth Centuries opened the door to modern medical science Mohler[2] has aptly pointed out that the bacteria viruses parasites, and toxins that cause diseases operate in accordance with established laws and that the knowledge gained of the causes of disease is not dependent on the nature of the academic degrees possessed by the research workers or practitioners who do the studies Rather we must agree that we are concerned instead with a worker s competence integrity and ability to apply the knowledge he develops Many valuable contributions to human medicine have been and will continue to be made by allied disciplines especially that of veterinary medicine

A study of the effect of the ingestion of vegetation and meat by animals may pave the way to a better understanding of the effect of such foods on man We must know the effect of vitamin or mineral deficient food or foods that have taken up an excess amount of chemicals from the soil when ingested by animals and humans This last is particularly true in this day when so many chemical poisons are being used Study of the effects of the ingestion of these foods and of foods contaminated with insecticides fungicides pesticides and drugs may give us a better understanding of the way in which food control should be developed

Spread of Disease

There is a need too to study the potential of the spread of disease today and in the future and to establish adequate methods to prevent or to control this threat Today when the most distant parts of the earth are only hours apart by air travel it is possible for a person or animal in the incubation stage of a disease to leave a country and arrive in another country half the world away before he manifests clinical symptoms of the disease This greatly complicates the problem of controlling infectious diseases

Disease control is further complicated by insects that spread from place to place in various ways Flies and ticks are carried on commodity shipments Lice mites flies and cattle grub larvae remain on animals during shipment The larvae of flies are transported in straw and manure Lice and ticks are spread by contact Moving livestock to new ranges or feed lots further spreads

and to share in the prevention of the problems of tomorrow that will result as man either adapts to or changes his environment

Summary

This paper discusses the relationship of animal health to human health and welfare The ways in which animal diseases affect man the uses that man can make of animals as he studies human diseases the reasons underlying difficulties in control and some new problems that man can expect to face are presented Finally it is pointed out that only the harmonious efforts of scientists and laymen can cope with the challenges to human health and welfare resulting from diseases in animals

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the epidemiology of disease with experiments to develop better and faster ways of identifying infectious agents and disease processes and with better methods of prevention treatment and rehabilitation

Problems Still Facing Man

Obviously a discussion of all the problems that must be studied is not possible but there are several areas of research and control that should be mentioned

Before going on to new programs we must make certain that the gains that have been made in disease control are maintained and extended

Every effort should be made to free our hemisphere of foot and mouth disease Eradication of rabies and brucellosis is a goal that can be achieved Therefore a program to extirpate rabies should be developed and the program to eradicate brucellosis should be accelerated

The control or eradication of the infectious hepatitides of man and animals of leptospirosis trypanosomiasis swine cholera mastitis and rinderpest the control of occupational diseases of animal origin elucidation of the epidemiology of the encephalitides and the control of arthropod borne diseases are some of the challenges facing scientists

As business and industry continue to decentralize their operations the problem of providing safe wholesome food from animal sources will become increasingly difficult because of the difficulties of supervision

Another problem confronting us that must be studied results from an advance in physical science the development of atomic energy The use of atomic energy poses special problems for biological scientists who must now consider and plan for the impact of this force with its radioactivity on human and animal health and food products particularly those of animal origin and for the control of waste products in proximity to humans and animals

Conclusions

The health of animals and the health and well being of humans cannot be separated The animal kingdom has influenced civilization through the ages and will continue to do so Since food and other products of animal origin are among man s basic needs animal diseases are a constant threat to the health and welfare of mankind therefore they must be controlled or eradicated as quickly as possible Once high standards of animal health are achieved they must be maintained as new efforts are exerted to extend and improve them

The control of disease in animals and man requires the integrated efforts of scientists of all disciplines working in harmony with the public health services the animal health agencies and the public information service

To assure an adequate control of each individual disease procedures should be developed to assure proper identification of the disease and the reporting of its occurrence to a central agency Studies should be developed to identify the weak links in the disease chain so that these may be broken and the disease eradicated All groups interested in animal health and in human health and welfare must join together in sharing attitudes knowledge information skills methods and plans to eradicate the great animal plagues that affect man

and to share in the prevention of the problems of tomorrow that will result as man either adapts to or changes his environment

Summary

This paper discusses the relationship of animal health to human health and welfare The ways in which animal diseases affect man the uses that man can make of animals as he studies human diseases the reasons underlying difficulties in control and some new problems that man can expect to face are presented Finally it is pointed out that only the harmonious efforts of scientists and laymen can cope with the challenges to human health and welfare resulting from diseases in animals

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Part I The Viral Encephalitides

INTRODUCTION

By William McD. Hammon
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The topic of this portion of this monograph, namely, viral encephalitides, requires some qualifying comment that should serve as a brief orienting introduction. As one who has worked a long time in this field, the provision of such a commentary is my prerogative and possibly the one useful contribution that I can make at this point.

"Viral encephalitides" is altogether too broad a term for what is to follow. It could include such varied maladies as rabies, mumps, herpes, lymphocytic choriomeningitis, and others. Perhaps a more inclusive term would be "arthropod-borne virus encephalitides." This term was introduced by W. C. Reeves and myself in 1943[1, 2] to take the place of a miscellany of names that seemed inadequate in view of the knowledge then available.

This name, now in common use for fourteen years, has, I regret to say, outlived its usefulness. Newer knowledge has indicated that dengue, yellow fever, Colorado tick fever, West Nile fever, and Sindbis fever are diseases not ordinarily associated with encephalitis in any recognized natural host and are closely related with the former group either antigenically or by other valid criteria. The agents in this broader group are now quite commonly called the arthropod-borne viruses, omitting the term encephalitis, and abbreviated as arbor viruses. This appears at first to be a reasonable step, but many of us have objections to the shorter term and particularly to its abbreviation. Use of the term arthropod-borne viruses is not restricted to viruses capable of producing human disease and including swamp fever of horses and many other maladies. I feel that the abbreviation is most unfortunate and I hope that its use will not spread. The objection is due to the fact that the abbreviation happens to form a Latin word and in international usage will certainly be misconstrued by many to have significance as a tree rather than as an abbreviation for arthropod-borne. Furthermore, a subgroup of which I am chairman of the Virus Subcommittee of the International Nomenclature Committee* is now searching for a suitable short pseudogeneric name such as poliovirus and myxovirus to apply to this group, and the concurrent use of arbor virus again would be confusing. The new officially accepted name may well turn out to be an abbreviation of arthropod and possibly zoonoses. One of the common features of this group of viruses is that they are not only arthropod-borne, but are dependent for survival in nature upon a viremic vertebrate host, generally one lower than man, with man usually serving only as an accidental host and not as the true reservoir. Thus they are viruses of

* The recommendations of this subcommittee appear in the *International Bulletin of Bacterial Nomenclature and Taxonomy* published by the Iowa State College Press, Ames, Iowa.

arthropod borne zoonoses not tree viruses or plain arthropod borne viruses Since they are for the most part if not entirely zoonoses (for example we are not certain regarding the endemic basic cycle of *Phlebotomus* fever and a few others but this may be parallel to the situation of yellow fever before jungle yellow fever was discovered) I am now inclined to suggest the epidemiological group name of arthropod borne zoonoses for as many as are known to qualify and to wait for the nomenclature committee to agree in the near future on a pseudogeneric short name probably with a Greek or Latin derivation

I feel it timely to point out that this monograph particularly this section of it deals principally with a few of our own domestic viruses that belong in this group of arthropod borne zoonoses These are only a very few of those known the world over a group in the process of antigenic classification by the hemagglutination technique that Jordi Casals and his colleagues at the Rockefeller Foundation virus laboratories New York N Y are now pursuing with results of great promise[3]

In the pages that follow are presented reviews of previous work and summaries of certain current work in this field by well selected investigators from the staffs of the Communicable Disease Center of the Public Health Service at several different installations and by the New Jersey State Health Department Trenton N J

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THE NATURAL HISTORY OF THE ARTHROPOD-BORNE ENCEPHALITIDES IN THE UNITED STATES

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Over a decade ago Bates (1946) pointed out the importance of the "natural history" viewpoint in investigating the mechanisms of maintenance and dispersal of a disease as compared with the sometimes more restricted concepts of "epidemiology" and "ecology." This is particularly true for diseases such as plague, yellow fever, and the encephalitides that have arthropod vectors and wild animal hosts as reservoirs. These diseases have enzootic cycles by which they are perpetuated in nature independently of their transmission to man. Determination of the basic factors responsible for their perpetuation requires coordinated field and laboratory investigations by individuals trained in many disciplines, including epidemiology, biology, ecology, and immunology.

In any field involving such a variety of disciplines it is inevitable that there will be differences of opinion with regard to terminology. In order to avoid confusion, the following definitions are given for some of the principal terms used in this paper:

Host: an animal (vertebrate or invertebrate) that is susceptible to infection with the disease organism (virus).

Inapparent host: an individual in which the virus produces no clinical manifestations of disease.

Apparent host: an individual in which the virus produces a clinical syndrome.

Reservoir: a host that serves as a source of infection for other hosts, including vectors.

Dead-end host: a host that does not serve as a source of infection for other hosts or vectors.

Vector: an arthropod or vertebrate that transfers infection from one host to another.

Sylvan vector: a vector that transfers infection from wild host to wild host (synonymous with "enzootic vector").

Endemic or epidemic vector: a vector that transfers infection to man and/or domestic animals.

Infection chain: the course of transmission of the virus from host to host (in temperate climates there are both summer and winter infection chains).

Transmission cycle: a cyclical infection chain in which transmission repeatedly follows the same type of course.

Basic transmission cycle: the infection chain through which the virus is normally perpetuated in nature.

Primary vectors or primary reservoirs: those that are essential elements in the basic transmission cycle or endemic chains of infection.

Secondary vectors or secondary reservoirs: those which are not essential elements in chains of infection, but which may sometimes be involved, particularly during epidemics or epizootics.

The authors are stationed in Logan, Utah, and Greeley, Colo., respectively.

Natural history of encephalitis the interrelationships of virus vectors, and hosts that are responsible for perpetuation of virus in nature and for its spread to man and domestic animals

Encephalitis (plural encephalitides) inflammation of the brain In the present paper the term encephalitis is used to refer specifically to arthropod borne viral encephalitis

In the United States there are three principal arthropod borne viruses that cause encephalitis in humans those of western equine (WEF) eastern equine (EEE) and St Louis (SLE) encephalitides As indicated by their names WEE and EEE produce clinical disease in horses as well as in man whereas SLE infections in horses are inapparent EEE also produces a clinical syndrome in birds and is a serious problem in pheasant farms in some of the eastern states

In recent years our knowledge of the distribution of these three viruses has been expanded considerably Prior to 1940 delineation of the geographical area of endemicity for each of these diseases was based largely upon the distribution of cases in man or in horses WEE was considered endemic only in the central plains and the Pacific coastal regions EEF was believed confined to the Atlantic coastal states Epidemics of SLE in man had been known only in the states along the upper Mississippi and lower Ohio valleys Since 1940 however our concept of the respective distribution of each of the three agents in nature has been considerably altered because of the isolation of virus from mosquitoes the detection of inapparent infection and the occurrence of clinical cases (FIGURE 1) We now know that although WEE occurs primarily west of the Mississippi River it is also present in some states along the Atlantic and Gulf coasts as evidenced by antibodies in vertebrate hosts and recoveries of virus from wild birds and mosquitoes (Holden 1955a Alexander and Murray 1957 Kissling *et al* 1955) SLE occurs in conjunction with WEE in the states west of the Mississippi River but it also has an important extension into the central states EEE occurs in the Atlantic and Gulf coast states from New Hampshire to Texas and as far inland as Wisconsin

It is believed highly improbable that these three diseases have spread to new areas during recent years Much more tenable is the belief that the infections have existed in the same general geographical areas since antiquity This does not mean that there have not been local extensions into areas in which the viruses were previously not present as for example in the millions of acres of formerly arid land in the West that have been brought under irrigation with resultant increases in the populations of bird reservoirs and mosquito vectors of encephalitis It is quite probable that further research will reveal an even wider geographical distribution for each of the three encephalitis viruses

There are certain basic similarities in the natural histories of the three types of encephalitis It is generally believed that birds may serve as natural hosts for all three and that various species of mosquitoes are vectors Humans and horses appear to be dead end hosts or at most secondary hosts for all three viruses Beyond these similarities there are important specific differences

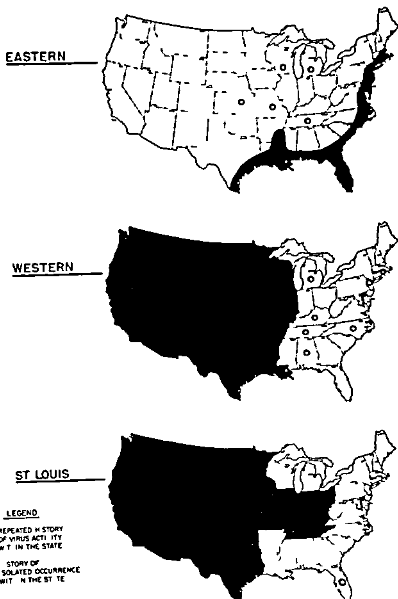


FIGURE 1 The geographical distribution of the arthropod-borne encephalitides in the United States Total virus activity in man and in animals

in the natural histories of the three diseases and the details of these differences are the topic of this presentation

Western Equine Encephalitis

Summer infection chains The summer infection chains for WEE are reasonably well established (FIGURE 2) There seems to be little question that

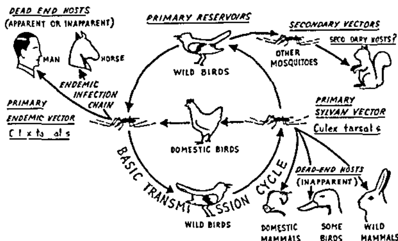


Figure 2 Summer infection chains for western equine encephalitis The chains for rural St Louis encephalitis are similar except that horses are inapparent rather than apparent hosts

Culex tarsalis is the primary vector in the sylvan transmission cycle as well as in the endemic chain of infection in the western United States It is the one species in which population densities virus infection and vector ability blood feeding habits and consistent association with epidemics are adequate to account for WEE encephalitis in humans and horses (Hammon and Reeves 1945 Reeves 1953 Eklund 1954 Cockburn Sooter and Langmuir 1957) Furthermore the distribution of this species in North America closely coincides with the general area in which outbreaks of WEE have been known to occur (Jenkins 1950) WEE virus has been isolated many times in nature from *C tarsalis* Isolations have also been made from *C pipiens* *C quinquefasciatus* *C restuans* *C stigmatosoma* *Aedes melanimon* (*dorsalis*) *A nigromaculis* *A infirmatus* *A vexans* *Anopheles freeborni* *Culiseta melanura* and *C inornata* as well as several species of bird mites and an assassin bug *Triatoma sanguisuga* (a general review of published records of isolations was made by Ferguson 1954 some later isolations were reported by Blackmore and Winn 1954 and Burroughs and Burroughs 1954 Reeves 1953 Kissling *et al* 1955) Species other than *C tarsalis* are however considered to be of secondary importance Some of them may however be essential in the transmission of WEE in the eastern and southeastern states since this is out of the range of *C tarsalis* *C pipiens* and *C quinquefasciatus* have been shown experimentally to be poor vectors for WEE (Hammon and Reeves 1943 Chamberlain *et al* 1954) The primary vectors of WEE in the East are therefore yet to be determined

Although it seems quite clear that birds are the important natural hosts of WEE (Hammon and Reeves 1945 Reeves 1955 Eklund 1954) it has not yet been determined which species are primary reservoirs and which are of secondary importance Published and unpublished records indicate that WEE virus has been recovered from about 20 species of birds and 6 species of mammals and that WEE antibodies have been found in more than 75

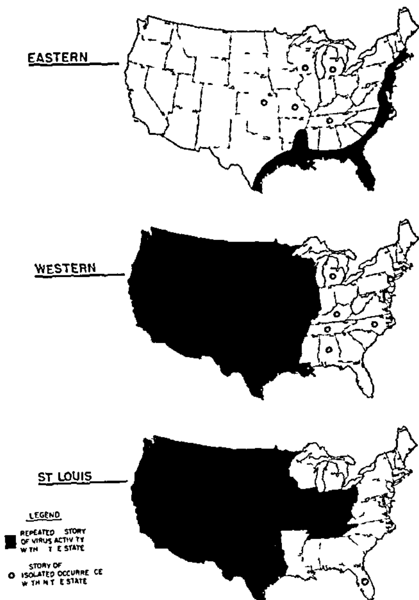


FIGURE 1. The geographical distribution of the arthropod-borne encephalitides in the United States. Total virus activity in man and in animals.

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Western Equine Encephalitis

Summer infection chains. The summer infection chains for WEE are reasonably well established (FIGURE 2). There seems to be little question that

increasingly to the importance of nestlings in natural chains of infection of the encephalitides

One possible explanation of the usual *tarsalis* bird feeding association is this many birds nest and roost in trees and as foliage gives off carbon dioxide at night when photosynthesis has ceased *tarsalis* which is attracted by carbon dioxide (Reeves 1951 Reeves 1953a) is thus brought into the sphere of attraction of roosting or nesting birds when it is seeking a blood meal Additional studies are under way at the Logan Field Station of the Communicable Disease Center (CDC) to evaluate the importance of carbon dioxide in the orientation of blood seeking *tarsalis* In atypical situations it would not be at all surprising to find mammals serving as the natural hosts for *tarsalis* For example Philip Bell and Larson (1955) found that almost 14 per cent of the black tailed jack rabbits in an arid area of northern Nevada had WEE antibodies thus suggesting a high degree of feeding by *tarsalis*

The longevity of the mosquito vectors and the extent of repeated blood feeding are important factors with regard to summer transmission cycles of encephalitis Obviously the mosquito must take an infective blood meal survive through the extrinsic incubation period and then take a second blood meal if it is to transfer infection from one vertebrate host to another In observations with *Aedes* mosquitoes Chamberlain Cornstan and Sikes (1954a) found that optimal transmission of WEE virus occurred in as short a period as 5 days and that high transmissibility was maintained for about a month It is difficult to obtain longevity data on mosquitoes under natural conditions but from the general information available it appears that a large percentage of summer populations of *C tarsalis* probably survives long enough to effect at least one transmission and under favorable conditions adults might survive long enough for several transmissions Current observations at the Bakersfield Cal Logan Utah and Greeley Colo field stations of CDC indicate that higher humidity in the adult mosquito s microhabitat greatly favors increased longevity of both summer and winter populations of *C tarsalis* Thus the vast irrigation developments in the western states may not only result in greatly increased production of *C tarsalis* but may also create conditions favoring increased mosquito longevity and therefore greater likelihood for the build up of epidemics of WEE encephalitis

As previously indicated the natural vertebrate reservoirs of encephalitis in a particular area must develop sufficient viremia for infection of the mosquitoes that transmit virus from host to host It has been shown in certain situations that *tarsalis* may feed as much on the larger mammalian dead-end hosts such as horses cattle and sheep as on birds (Reeves and Hammon 1944) Where there is an abundance of these dead-end hosts in relation to avian hosts they may deflect blood seeking vectors from the human population (zooprophylaxis) decrease the extent of feeding upon infected avian reservoirs and drain off virus that otherwise would reinforce the sylvan avian cycles of encephalitis transmission Thus the chances of human infection would be decreased both by the lower infection rates in mosquitoes and by their lower attack rates upon humans Potentialities for this type of zooprophylaxis exist in a number of

species of wild birds and a half dozen species of wild mammals as well as most of the common domestic birds and mammals The problem then is to determine which of this bewildering array of species are essential reservoirs for the enzootic perpetuation of WEE under natural conditions and whether others may form necessary links in endemic chains of infection

R W Chamberlain R E Kissling and their co workers have done some highly significant research on encephalitis virus vector and virus host relationships under controlled laboratory conditions as described elsewhere in this monograph Such studies are invaluable in establishing potential vector host combinations that may be important for the perpetuation of encephalitis viruses in nature However the mere fact that a particular vector has a sufficiently low infection threshold to become infected by biting a host that develops a certain level of viremia does not mean that this vector host combination is important in nature An analogous situation exists with regard to malaria in that most *Anopheles* mosquitoes can develop and transmit malaria plasmodia in laboratory tests however relatively few *Anopheles* species actually are vectors under natural conditions In addition to having the required vectoring and reservoiring potentials the primary vectors and reservoirs of encephalitis must be appropriately associated in both time and space

Recent observations indicate that *Culex tarsalis* exhibits a peak of biting activity at dusk whereas the *Aedes* mosquitoes in the same area reach biting peaks earlier in the evening (Beadle 1955) It may be epidemiologically significant that the fiercely biting *Aedes* cause many people to seek shelter within their screened dwellings before they are exposed to the principal biting attack of *C tarsalis* a primary vector of encephalitis Since *C tarsalis* has its peak biting activity at dusk this should be a critical period in the transmission of WEE virus and the primary reservoirs of WEE are probably among the hosts that are available to *C tarsalis* at this time of day It is a common observation of field workers that the evening flights of birds to their nocturnal roosting sites take place a short while prior to this peak of the biting activity of *tarsalis*

Precipitin and other tests with field collected mosquitoes usually indicate that *tarsalis* feeds largely on birds but there is ample evidence that it also feeds on a variety of mammalian and even reptilian hosts (Reeves and Hammon 1944 Dow Reeves and Bellamy 1957) Our general observations including unpublished studies by R P Dow indicate that *tarsalis* does not necessarily prefer to feed upon birds but that it will feed on mammals as readily as on birds if both are equally available It also appears that the inherent behavior characteristics of the available hosts particularly their degree of quiescence are probably more important than the particular species that are at hand For example unpublished observations by J S Blackmore and R P Dow show that engorgement rates for *C tarsalis* were much higher on relatively inactive nestlings than on the more active adult birds The parser vesture of the nestlings may also have been a factor in the higher feeding rates It is generally recognized that embryos and very young animals are more susceptible to infection with encephalitis viruses Thus cumulative evidence points

1955 Reeves *et al*, 1955) and it is generally agreed that mites do not play any significant role in the perpetuation of WEE encephalitis virus in nature

(3) There is much evidence to support the possibility that WEE virus may pass the winter in mosquitoes W C Reeves and his co-workers (in press) have isolated WEE virus from naturally infected *Culex tarsalis* in Kern County Calif during all months of the year except December and Blackmore and Winn (1956) isolated the virus from *C tarsalis* collected in Colorado during December Bellamy Reeves and Scrivani (in press) recovered WEE virus from experimentally infected *C tarsalis* after the mosquitoes had been kept for 113 days in an unheated cellar during the winter *C tarsalis* completed extrinsic incubation and transmitted WEE virus to chickens after 97 and 109 days of winter incubation

Two aspects of the biology of *C tarsalis* are of particular importance in relation to its potential role as an overwintering reservoir for WEE virus namely its overwintering habits and its prehibernation feeding habits *C tarsalis* may be found during winter months in abandoned mines and cellars (Keener 1952) There is indication however that the temperatures in most such shelters are too high for successful hibernation and the normal winter habitat for this mosquito in the more northern latitudes has yet to be discovered (Dow Mail and Richards 1956)

If *Culex tarsalis* is to serve as an overwintering reservoir for encephalitis viruses it must have an infective blood meal before going into hibernation There is however strong evidence of a prehibernation decline in blood feeding by *tarsalis* with a possible switch to carbohydrate sources of food (Mail and Smith in press) There is even a possibility that a prehibernation blood meal may be detrimental to overwinter survival of the mosquito (Hammon Reeves and Galindo 1945) These relationships of prehibernation feeding to overwintering of *C tarsalis* obviously would decrease its potentialities as an over winter reservoir for virus Investigations to obtain additional information on these relationships are now under way at the Greeley Logan and Bakersfield field stations of CDC

(4) There is also strong experimental evidence to support the possibility that WEE virus may pass the winter in resident avian hosts W C Reeves R E Bellamy and G A Hutson have conducted extensive investigations in Kern County Calif that show that WEE virus can be recovered from experimentally infected birds after extended periods that are considerably greater than the normal hibernation period for the mosquito vectors The results of these studies are now being summarized for publication

Some virus diseases such as those of psittacosis and herpes simplex have extended periods of latency Observations on some of the encephalitis viruses also support the possibility that there may be long periods during which the agent remains in the host in a latent or avirulent condition For example Corristan LaMotte and Smith (1956) showed that bats infected with Venezuelan equine encephalitis and stored at hibernating temperatures (10 C) maintained a low order viremia for at least 90 days and that the viremia level rose rapidly when the animals were returned to room temperatures This suggests that latent virus of other encephalitides might be reactivated in verte

irrigated areas in the West, where dairying and the raising of cows and other domestic mammals constitute the predominant agricultural industries. To our knowledge, however, there has never been any attempt to correlate mosquito and bird infection rates in these areas with population densities of cattle or other dead end hosts of encephalitis.

The primary reservoirs in the sylvan cycle of WEE encephalitis may not necessarily be the same as those involved in the chain of transmission to man and domestic animals. For example, in a particular situation sylvan WEE encephalitis might be perpetuated continuously by an association of *C. tarsalis* with blackbirds without any involvement of human infections. The addition to this complex of a more domestic avian host, such as the English sparrow, however, might result in a build up of infection rates in populations of *tarsalis* associated with man and thus lead to human infection.

With a given mosquito infection rate, the chances for human infection will be correlated directly with the vector's biting or attack rates on man. These attack rates are obviously related, in turn, to mosquito population densities. Recent studies with caged birds indicate that the percentage of *tarsalis* feeding on avian hosts decreases as the number of mosquitoes increases (Dow, Reeves and Bellamy, 1957). This would suggest that at times of unusually high mosquito prevalence there may be a much greater deflection of *tarsalis* from avian hosts to mammals, including man and domestic animals. Similarly, increased human attack rates might result from a decrease in population of avian hosts despite the fact that the mosquito population remained constant.

From the above discussions it is apparent that, whereas the identity of the primary vector of WEE seems to be reasonably well established, much more information is needed on the particular avian species that serve as primary summer reservoirs and on the critical ecological relationships between hosts and vectors. Such information is needed if we are to understand the causative mechanisms of WEE epidemics.

Winter infection chains of WEE. In temperate North America, where vector populations are inactive during the winter months, it is obvious that there must be some reservoiring mechanisms that enable encephalitis viruses, in the absence of active transmission, to survive this period. Five possibilities have received investigative attention: (1) transovarian passage, (2) overwintering in infected mites, (3) overwintering in infected mosquitoes, (4) overwintering in infected birds, and (5) reintroduction each spring by migrating birds. The potentialities for each of these mechanisms with regard to WEE will be discussed separately.

(1) Transovarian passage in ticks is recognized as a mechanism for the perpetuation of louping ill and of Russian spring summer encephalitis. Since the primary vectors of WEE appear to be *Culex* mosquitoes that overwinter as adult females, the possibility of transovarian passage as an overwintering mechanism for this particular virus seems to be eliminated.

(2) WEE virus has been isolated from the mites of wild birds (Reeves *et al.*, 1947; Miles *et al.*, 1951) and for a time it was believed that they might constitute the sylvan vector as well as a long term reservoir. Recent laboratory investigations, however, have given negative results (Chamberlain and Sikes

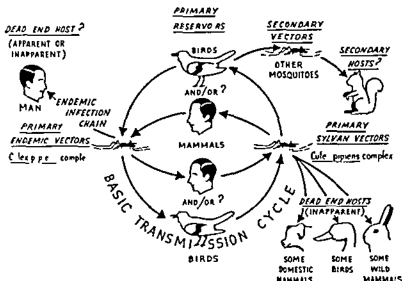


FIGURE 3 Summer infection chains for urban St Louis encephalitis The chains for rural SLE are similar to those for WEE (FIGURE 2)

1954 Reeves 1953) but most isolations have been from *C tarsalis* or the *C pipiens* complex which appear to be the primary rural and urban vectors respectively

As in the case of WEE present information is insufficient for determination of the particular species that are primary reservoirs of SLE Until recently SLE virus had not been isolated from any wild or domestic birds (Eklund 1954) but has now been recovered from several species (Kanzenhofer *et al* 1957) SLE neutralizing antibodies have been found in most of the common domestic birds and mammals as well as in about 55 wild birds and a half dozen wild mammals The problem of determining which of these many species are primary reservoirs is therefore similar to that for WEE For SLE however the problem is further complicated by the possibility that the primary reservoirs for rural and urban SLE may be different Birds may serve as natural reservoirs for urban SLE but it is known that they develop relatively low viremia levels with this virus (Hammon Reeves and Sather 1951 Chamberlain *et al* 1957) These levels are sufficient to infect the more susceptible mosquito species such as *C tarsalis* but the extent to which the less susceptible *C pipiens* complex may become infected by feeding on birds has not been fully determined The possibility that mammals may be involved as reservoirs of urban SLE cannot be overlooked It is known that other arthropod borne encephalitis viruses may have mammalian reservoirs For example Venezuelan equine encephalitis will produce viremia levels in bats well above the threshold levels for infection of some mosquito species (Corristan LaMotte and Smith 1956) Observations on the recently discovered Kasanur Forest disease in India suggest that monkeys may serve as the natural reservoir

brates or arthropod hosts at times of physiological change as, for example during the mating and egg laying season of avian hosts or when mosquitoes emerge from hibernation

(5) Reintroduction of encephalitis virus each year into endemic areas by avian hosts migrating from tropical areas of continuous transmission offers a possible means of overwintering for which there is some circumstantial evidence (Kissling *et al* 1955) With regard to WEE however it is generally believed that resident avian hosts or arthropod vectors are the more likely winter reservoirs at least in the Far West On the basis of their observations in Kern County to date Reeves Bellamy and Scrivani (in press) conclude that The possibility that a vertebrate host is the immediate source of winter time vector infection is as likely as the alternative that virus survives solely in the vector in winter

St Louis Encephalitis (SLE)

Summer infection chains of SLE In the Far West it seems apparent that the summer transmission cycle for SLE in rural situations is similar to that for WEE *C tarsalis* serving as the primary vector and birds as the natural vertebrate reservoir (FIGURE 2) The basic ecologic interrelationships for rural SLE would thus be similar to those outlined for WEE but this does not imply that there are not important differences between the two One such difference is that WEE outbreaks usually occur in midsummer whereas SLE outbreaks take place in the late summer or fall even when the two occur in the same geographical area This might be due to longer intrinsic or extrinsic incubation periods for SLE which in turn would limit its build up in the shorter summer seasons at higher altitudes or more northerly latitudes Such inherent differences in the two agents may require different specific interrelationships between vectors hosts and viruses despite the fact that their natural chains of infection are similar

Although the infection chains for WEE and rural SLE appear to be quite similar there is increasing evidence that the transmission cycle for SLE in urban situations of the central states and possibly other areas is quite different (FIGURE 3) The *Culex pipiens* complex is being increasingly implicated as the primary vector for urban SLE The importance of these household mosquitoes was first postulated by Lumsden (1934) and was based on his investigation of the 1933 epidemics in the vicinities of St Louis and of Independence Mo Subsequent findings of other workers have further supported Lumsden s original hypothesis The *C pipiens* complex in the United States includes *pipiens molestus quinquefasciatus* and possibly others (Mattingly *et al* 1951) Under laboratory conditions members of this complex have been known for some time to be capable of transmitting SLE virus High population densities of one or more species have been found in vicinities in which SLE epidemics have occurred and collections of these mosquitoes from localities in which epidemics were in progress have yielded specimens from which SLE viruses were isolated (Kanzenhofer *et al* 1957 Beadle *et al* 1957)

In addition to the *C pipiens* complex and *C tarsalis* SLE virus has been isolated in nature from *C stigmatosoma* and *A melanimon (dorsalis)* (Ferguson

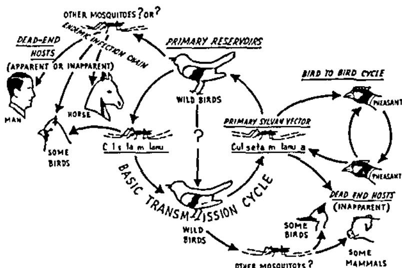


Figure 4 Summer infection chains for eastern equine encephalitis

siana Five recoveries of EEE virus have been reported from *C melanura* in New Jersey (Holden Miller and Jobbins 1954 Burbutis and Jobbins in press) Isolations of EEE virus in nature have also been made from *Mansonia perturbans* (Howitt *et al* 1949) *Anopheles crucians* (Kissling *et al* 1955 Karstad *et al* 1957) *Culex salinarius* (Burbutis and Jobbins in press) *Aedes mitchellae* and a pool of *Culicoides* that had not been identified as to species (Karstad *et al* 1957) Laboratory attempts to transmit EEE virus with *C salinarius* and *A crucians* have not been successful (Chamberlain *et al* 1954) The geographical distributions of *A mitchellae* and salt marsh species of *Culicoides* in the United States coincide in general with the distribution of EEE and these species merit further attention as potential vectors of EEE

The distribution of salt marsh mosquitoes *Aedes sollicitans* and *A taeniorhynchus* is also similar to that of EEE and circumstantial evidence would indicate that they might be involved as endemic vectors of EEE as for example in the outbreak which took place in the Dominican Republic in 1948 and 1949 (Eklund Brennan and Bell 1950 Eklund Bell and Brennan 1951) In laboratory tests with *A sollicitans* Chamberlain *et al* (1954) have given this species a vector potential rating of excellent

In assessing the importance of *C melanura* as a vector of WEE and EEE viruses it is necessary to take into account that this mosquito has rarely been observed to bite man even in situations where this insect was known to be present in considerable abundance Further information is needed on the feeding habits of *C melanura* If it truly does not bite man the endemic vector of EEE must be another species of mosquito or some other arthropod

from which this Group B type virus is transmitted by arthropods to man (Work and Trapido 1957) It is also well established that mammals are natural reservoirs of Russian spring summer encephalitis and other arthropod borne virus diseases such as yellow fever and dengue Further study may indicate that mammals (including man) may possibly serve as reservoir links in SLE chains of infection

The *C pipiens* complex is quite variable biologically and the different variants are quite labile and difficult to distinguish taxonomically (Mattingly *et al* 1951) For example, true *pipiens* probably does not feed upon mammals, whereas *molestus* even though it is autogenic may feed avidly upon man and other mammals Further work on the summer transmission cycles for urban SLE must therefore include detailed studies on the feeding habits of the *C pipiens* complex as well as studies of the vertebrate hosts

In situations where small urban communities are surrounded by irrigated rural areas it is possible that there may be mixed epidemics of rural and urban SLE in which both *C tarsalis* and the *C pipiens* complex are involved as primary vectors This may have been true for the SLE outbreak in the Texas Panhandle during 1956 (Alexander and Murray 1957) Studies during previous years had shown that both *C tarsalis* and *C quinquefasciatus* were abundant in the area of the outbreak (Harmston *et al*, 1956) and SLE virus has been recovered from both these species in several other areas

Winter infection chains of SLE The possible winter reservoirs for *C tarsalis* transmitted rural SLE appear to be quite similar to those for WEE The winter reservoir mechanisms for urban SLE may also be similar to those for WEE, although involving different primary reservoirs and vectors Positive evidence on the possibility that hibernating mosquitoes serve as winter reservoirs for SLE has been obtained by Bellamy Reeves and Scrivani (in press) who found that SLE virus survived for 116 days in experimentally infected *C quinquefasciatus* that were kept in an unheated cellar during the winter months Hurlbut (1950) has also shown that *C quinquefasciatus* can carry the virus of Japanese B encephalitis through a 91 day period of simulated hibernation and retransmit it to young albino mice

Resident birds are also possible winter reservoirs for SLE Chamberlain and his co workers (1957) recovered SLE virus from the gizzard of a cowbird 38 days after infection

The likelihood that migrating birds may reintroduce SLE virus into some endemic areas of the United States each spring requires further investigation

Eastern Equine Encephalitis (*EEE*)

Summer infection chains of EEE The summer infection chains for EEE are quite different from those for WEE and SLE and some of the important links are yet to be discovered (FIGURE 4) The identity of the primary vectors is particularly puzzling Most of the accumulated evidence suggests that *Culiseta melanura* is the primary sylvan vector responsible for bird to bird transmission in nature Chamberlain *et al* (1951) reported one isolation of EEE virus from naturally infected *C melanura* mosquitoes collected in Loui

function among wild bird species in their natural habitat However unless the mode of transmission once demonstrated is shown to be peculiar to the activities of confined pheasants it should be further considered as a possible means of survival for the virus in nature (FIGURE 4)

Winter infection chains of EEE Of the three agents under discussion EEE virus has been studied least in regard to its mechanism of survival during the winter months To our knowledge no detailed studies have been made on the ability of any arthropod to serve as the winter reservoir *Culiseta melanura* the mosquito thought to be the principal sylvan vector is thought to hibernate in the larval stage This belief is based upon the finding of viable larvae under winter conditions but there is no specific information available on the fate of the adult at the end of the active mosquito season

The possible role of birds in the overwintering of EEE virus likewise has received only limited study There are no published reports of attempts to demonstrate latent virus in birds that previously had been infected with the agent Kissling *et al* (1955) reported on an attempt to test the hypothesis that EEE and WEE virus were introduced into the United States annually by migrating birds These workers found a higher antibody rate against each agent in birds migrating into the tropics during the winter months than in permanent or winter resident birds in Louisiana Although the results of this study lend support to the hypothesis that the equine viruses are reintroduced each year by migrating birds the evidence is by no means conclusive

Conclusion

In looking back over the investigations of encephalitis of the past decade it appears that perhaps too little attention has been given to the vertebrate reservoirs in nature In our preoccupation with the arthropod vectors we have frequently neglected the vertebrate hosts Probably our thinking in this regard has been too much conditioned by experiences with other mosquito borne diseases such as malaria and yellow fever In the western states at least there has been a recent trend toward giving more research attention to the vertebrate hosts Eventually we may find that for the arthropod borne encephalitides an attack upon the primary reservoirs is more feasible than vector control in breaking the chain of infection It is important therefore that we give full consideration to all of the critical interrelationships in the natural history of these diseases if we are to accomplish our ultimate objective of prevention or control of encephalitis

Summary

The three principal arthropod borne viruses in the United States are western equine (WEE) eastern equine (EEE) and St Louis (SLE) WEE and SLE occur primarily in the twenty two western states whereas EEE occurs primarily in the Atlantic and Gulf coast states Birds serve as natural hosts and mosquitoes as vectors for all three viruses Man and horses are accidental hosts Clinical disease in man is produced by all three viruses in horses by WEE and EEE and in birds by EEE alone

that feeds on man and horses as well as on birds Fortunately for man mosquitoes of the *C pipiens* complex are poor vectors of EEE (Davis 1940 Chamberlain *et al* 1954) and are probably not involved as endemic vectors

Information on the natural hosts of EEE is even more meager than for WEE and SLE A few recoveries of EEE virus have been made from wild and domestic birds and mammals and antibodies have been found in a wide variety of species including 40 or 50 kinds of birds Laboratory studies with EEE infections in horses (Kissling *et al* 1954) indicate that they develop relatively low viremia levels and it seems most likely that birds serve as the primary reservoirs in the basic infection chain The specific identity of these reservoirs is yet to be established Some birds such as red winged blackbirds cardinals and English sparrows may be apparent hosts in which EEE infections are almost always fatal others such as American egrets snowy egrets and white ibis appear to be inapparent hosts (Kissling *et al*, 1954a)

In summary it seems reasonable to assume that the enzootic foci for EEE are fresh water swamps that harbor the wild bird reservoirs and in which the primary sylvan vector *C melanura* breeds The endemic chains of infection through which EEE is carried from these enzootic foci to man and domestic mammals are however yet to be determined (Holden 1954)

EEE infections in ring necked pheasants In the process of elucidating the natural histories of many diseases epidemiologists have frequently observed phenomena for which no explanation was readily available on the basis of pre existing knowledge or belief For example it was originally thought that the infection chain of yellow fever consisted solely of a man mosquito-man trans mission cycle Furthermore *Aedes aegypti* was believed to be the only vector It was subsequently observed however that the causal virus survived admira bly in areas where man was infected too infrequently to serve as the reservoir and/or where the known vector had been effectively controlled These ob servations led to the discovery of transmission cycles in which man and *Aedes aegypti* had no part From these findings have evolved the now well known concepts of so called urban and jungle yellow fever (Bates 1946)

The natural history of the encephalitides is also complicated by findings that are not satisfactorily explained by the hypotheses that have been discussed The results of studies on the epizootiology of EEE in confined flocks of ring necked pheasants (*Phasianus colchicus torquatus*) are illustrative of findings that are inconsistent with the hypothesis that the encephalitides are invariably mosquito borne

Reports on the behavior of the disease in naturally occurring outbreaks in pheasants (Beaudette and his co workers 1939 1941 1945 1948 and 1952) and under controlled experimental conditions (Holden 1955) provide conclusive evidence that the infection may be spread in these hosts by some means of direct contact that does not involve transmission by arthropods We can only speculate upon the significance of these findings It is realized that the ring necked pheasant is not native to those areas in which EEE is epizootic Furthermore studies have been made only on flocks reared in captivity Thus it is possible that the mechanisms of spread among captive pheasants cannot

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Wild birds serve as the primary reservoir and *C tarsalis* as the primary sylvan and endemic vector, for WEE and rural SLE Mosquitoes of the *C pipiens* complex appear to be the primary sylvan and endemic vectors of urban SLE mammals as well as birds are suggested as possible reservoirs Wild birds also are primary reservoirs for EEE and *C melanura* seems to be the primary sylvan vector the endemic vector for EEE is yet unknown Direct bird to bird transmission of EEE among pheasant flocks has been proved, but its possible role among wild birds has not been determined

Either the mosquito vectors or the avian hosts appear to be possible over wintering reservoirs for encephalitis viruses Studies with WEE and SLE have shown that both of these viruses can survive for extended periods in either mosquitoes or birds The possible reintroduction of virus into endemic areas each season by migrating birds requires further investigation Over wintering in mites or transovarian passage is not believed to be the mechanism for the winter survival of encephalitis viruses

Ecological interrelationships in time and space determine the actual role of potential vectors or hosts in the epizootiology and epidemiology of encephalitis Of primary importance are the population densities distribution and nesting resting and roosting habits of the avian hosts and the population densities summer and winter longevity blood feeding habits and host preferences of the arthropod vectors An abundance of available dead end hosts such as domestic mammals may provide a considerable degree of zooprophylaxis for the human population

It is believed that the role of specific vertebrate hosts in the natural history of the encephalitides deserves greater investigative attention and that control of vertebrate reservoirs as well as vector control offers promise in preventing or limiting epidemics of encephalitis among man and domestic animals

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VECTOR RELATIONSHIPS OF THE ARTHROPOD-BORNE ENCEPHALITIDES IN NORTH AMERICA

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Although much remains to be learned about the ecology of the arthropod borne encephalitides in North America, past and recent studies have provided considerable knowledge of these diseases Infection in horses and man has been associated with large mosquito populations and infection in wild birds and birds have been shown to be important hosts [1, 9] Many arthropod species have been tested in the laboratory for their susceptibility to virus infection and for their ability to transmit and certain mosquitoes associated with birds have been indicted as vectors [1, 9, 12] Antibody and virus isolation studies in wild vertebrates have indicated the field host ranges and virus isolations from field caught mosquitoes have revealed those species that actually feed upon infected animals under natural conditions [1, 3, 6, 9, 11, 14-1] Further information on the feeding habits of mosquitoes has been gained through field and laboratory observations and precipitin tests on naturally acquired blood meals Correlation of mosquito susceptibility with viremias attained in various vertebrates has furnished clues concerning favorable host vector combinations [10] These are only a few of the important contributions that have been made

As a result of these contributions certain vector relationships now appear reasonably clear By making the most of this knowledge a logical selection of the most probable vectors is possible

Eastern equine encephalitis (EEE) Field investigations suggest strongly that the foci of EEE infection are fresh water swamps Outbreaks of EEE in horses or man have generally originated in the vicinity of swamps and the presence of the virus in birds and mosquitoes during intraepidemic and intra epizootic periods has been established in swampy areas A high proportion of swamp inhabiting birds becomes immune during the transmission season and numerous isolations of virus have been made from their blood [8, 15, 16, 1, 3] Furthermore laboratory studies have shown wild birds to be highly susceptible to infection and to produce levels of virus in their blood sufficiently high to infect certain species of mosquitoes [15, 4] Complete bird mosquito bird transmission cycles have been accomplished in the laboratory

Attempts to isolate EEE virus from horse and man biting mosquitoes and other insects during epidemics or epizootics have almost always met with failure although thousands of individuals of such common species as *Aedes sollicitans A vexans A triseriatus* and *Psorophora ferox* have been tested On the other hand tests on a swamp breeding mosquito *Culiseta melanura* have been highly productive Eleven isolations of the virus have been made from this species in Louisiana [18] New Jersey [11, 19] and Massachusetts [5] always in association with active infection in the swamp-frequenting bird population This species has been shown in the laboratory to be susceptible to infection and to transmit by its bite [10]

A lesser number of isolations has been made from other species of mosquitoes. *Mansonia perturbans* collected in Georgia yielded virus on one occasion.[28] As it is also a swamp breeder, will feed upon birds, and can transmit with moderate efficiency in the laboratory,[10] it falls under suspicion as a possible vector. *Anopheles crucians* has yielded virus on two occasions but only in trace amounts.[14, 27] The importance of these isolations is negligible since this species has been shown to be incapable of transmitting infection in the laboratory.[10] Virus has been isolated twice from blooded *Culex salinarius* taken in a swamp.[11] This species has also been shown to be refractory to EEE infection in the laboratory,[10] and, as the infection rate of associated birds was high at the time the isolation was made, the virus detected was most likely in recently ingested bird blood. One isolation has been made from *A. mitchellae* and another from a pool of *Culicoides*, both apparently associated with infection in horses.[27]

The virus isolations from *Culiseta melanura* are made more significant by *the fact that relatively few specimens were tested.* *An isolation has been* made from about every 200 to 400 specimens checked, despite the fact that they were collected near their breeding sites and that young previously unfed individuals made up a high proportion of the catches.

Culiseta melanura is known to breed only in fresh water swamps or in heavily shaded seepage areas swamplike in character. Although it is a little known species, it is common in its preferred breeding areas. While it has been found as far west as Colorado and Oklahoma,[29] its range is generally restricted to the eastern half of the United States and coincides with the distribution of EEE in this country. Precipitin tests on specimens engorged with blood when *captured have shown that birds are preferred hosts.*[11, 22]

The evidence at hand points, therefore, to fresh water swamps as enzootic foci, to birds as primary hosts, and to *C. melanura* as the principal enzootic vector. In view of the restricted habitat of *C. melanura*, however, and its apparent preference for bird hosts, epizootics in horses and the occasional cases in man cannot logically be attributed to this species. It seems more likely that other vectors are required to carry the infection from birds to horses and to man.

The infrequency of infections in man rules him out as a likely source of infection for more mosquitoes.[9, 20] Horses likewise appear to be of limited *importance in epizootic spread of the disease* since they rarely produce enough virus in their blood to infect mosquitoes efficiently.[21] They apparently receive their infections mostly from the bites of mosquitoes that have fed previously upon birds.

In view of these restrictions, certain conditions appear necessary to permit an epizootic in horses (and an epidemic in man) to occur. Bird infection would need to be at high level, furnishing a relatively great source of virus for mosquito infection. This condition would depend upon an adequate population of *Culiseta melanura* (and assisting species such as *Mansonia perturbans*) and a low immunity rate in the bird population. The latter would depend in turn *upon a light infection rate the previous year.* As some of the infected birds fan out into the adjacent areas, they become diluted with noninfected birds

not associated with the swamp foci This, together with the fact that a relatively small proportion of the common horse feeding mosquitoes find opportunity to feed upon birds, make it necessary that the population of these mosquitoes be high If, in addition to all this, there is a low immunity rate in the horse population conditions are favorable for the epizootic Now with large numbers of the epizootic species of mosquito present, the likelihood of additional horse infections through mosquitoes infected from an occasional horse with higher viremia becomes possible[20] A high horse infection rate also favors occasional mechanical transmissions from horse to horse by the interrupted feedings of biting insects such as *Culicoides*[27] horseflies, deer flies, stable flies and mosquitoes That mosquitoes and stable flies are capable of transmitting mechanically between laboratory animals has been shown experimentally[11]

Mechanical transmission between caged pheasants by means of pecking has been demonstrated[33] There is no proof however that this mode of transmission is of much consequence among wild, unconfined birds It seems unlikely to influence the natural cycle of the disease appreciably

Western equine encephalitis (*WEE*) The ecology of this disease has undergone intensive investigation over many years[1,9,14] Hundreds of isolations of the virus from *Culex tarsalis* attest to the close association of this mosquito species with natural sources of infection Serologic and virus isolation studies in wild birds have firmly established their role as the most important hosts Inoculation of wild birds in the laboratory has confirmed their susceptibility and revealed viremias adequate to infect susceptible mosquitoes[34,35] Transmissions to barnyard fowl[36] and wild birds[35] by mosquitoes have also been accomplished in the laboratory

Studies on the feeding habits of *C tarsalis* have shown that while this species feeds most frequently upon birds it is also attracted to man horses and numerous other vertebrates *It is closely associated with birds in the West* for it breeds in natural temporary water sources in dry land areas and in irrigation water overflows in cultivated areas the same sites in which many bird species concentrate Because of its numbers its close association with birds its avidity for man and horses and its great susceptibility as proved in laboratory tests *C tarsalis* fulfills requirements for both an endemic and an epidemic vector While many other species of mosquitoes are susceptible particularly the *Aedes* very few isolations of virus have been made from them It is possible that *A dorsalis* and *A nigromaculis* which are abundant during the encephalitis season might assist in the spread of WEE in horses once an outbreak is under way in the same manner that certain *Aedes Psorophora* or other genera may spread EEE infection from an occasional high viremia horse At most however this mode of spread would appear to be only supplementary to transmission from birds to horses by *C tarsalis* as WEE viremias in horses are apparently even lower than EEE viremias[37] Occasional transmissions from birds to horses by *Aedes* probably also occur but with considerably less frequency than by *C tarsalis* because of the differences in their bird feeding habits

Until a few years ago the distribution of WEE was believed to be limited to *Culex tarsalis* Now we know that this is not the case While disease in

horses and man appears generally to be limited to areas where *C tarsalis* is the vector infection in wild birds and mosquitoes in swamps in the East are known to occur in the absence of *C tarsalis* In 1952 several isolations of WEE virus were made from birds collected in and adjacent to a swamp in Louisiana this same survey[16] also yielded an isolation of this virus from *Culiseta melanura* and two others from *Aedes infirmatus* a species breeding on the swamp margin In 1953 Holden[33] isolated WEE virus from a sparrow collected adjacent to a swamp in New Jersey, and Kissling[8] detected WEE antibody in resident birds and horses in Massachusetts An isolation of WEE from freshly engorged *C pipiens quinquefasciatus* in North Carolina was made in 1955[11] This isolation established the presence of WEE in that area The importance of this mosquito species as a vector is greatly in doubt however as it has been shown to be refractory to WEE infection in the laboratory[10] The virus isolated was undoubtedly from the blood recently ingested and did not represent true mosquito infection In 1956 several WEE isolations were made from the blood of birds collected in and near a swamp in New Jersey[21] Again a WEE virus isolation was made from *C melanura*[11]

This evidence indicates that the enzootic status of WEE in the East is similar to that of EEE Its unimportance as a disease producing agent in man and horses is probably due chiefly to the absence of its highly efficient vector of the West Lower viremias in birds are produced than by EEE making infection of various *Aedes* and other horse and man feeding mosquitoes that may occasionally feed upon birds less likely Also the viremia of WEE is lower in horses than that of EEE[27] so that horse-to-horse transmission must be an even more rare occurrence In addition laboratory studies have shown that when animals immune to EEE were infected with WEE they produced significantly lower viremias than nonimmune animals[24] This factor may be important in areas where a high proportion of birds and horses possess EEE antibody A combination of these deterrent effects probably serves to keep WEE within it enzootic foci in the East

St Louis encephalitis (SLE) Strong evidence indicates that SLE like EEE and WEE is primarily a bird disease transmitted by mosquitoes[1, 6, 9, 34, 39] Apparently similar factors foster its successful propagation namely adequate populations of susceptible birds and vector mosquito species and close association between the birds and the vectors Again man seems to be an unfortunate accidental host

The virus of SLE differs from those of EEE and WEE however in having an almost completely reversed mosquito susceptibility range EEE and WEE viruses develop readily in all species of *Aedes* and *Psorophora* thus far tested but do so poorly or not at all in most *Culex* except *C tarsalis*[10, 13] SLE virus on the other hand is highly infectious for all species of *Culex* that have been tested but does not develop well in *Aedes* and *Psorophora*[11, 13] These vector differences are reflected in the epidemiology of SLE not in the West where *C tarsalis* abounds but farther east in the central states where *tarsalis* is scarce or absent

Extensive studies in the Far West particularly in the Central Valley of California have shown with little doubt that *C tarsalis* is the main SLE

vector in that area and is responsible for most of the transmission to wild birds domestic fowl and man. Up to 1953 69 isolations of the virus had been made from wild caught *C. tarsalis* as against only 3 for *C. quinquefasciatus*, 2 for *C. stigmatosoma* and 1 for *A. dorsalis*.[3] In 1954 alone 87 additional isolations from *C. tarsalis* were reported.[17] The greatest outbreaks have coincided with an abundance of *C. tarsalis* and a high proportion of the cases have been rural rather than urban.[40] As might be expected the epidemiology is similar in many respects to that of WEE.

A different epidemiological picture has been presented in the central and east central states, namely, in the outbreaks in St. Louis, Mo., 1933, Hidalgo County, Texas, 1954, Calvert City, Ky., 1955 and Louisville, Ky., 1956. In those areas *C. tarsalis* was scarce or absent. There the human cases were mostly urban or suburban, and they were associated with heavy breeding of *C. pipiens* or *C. quinquefasciatus*.[5, 6, 20, 25, 41] These mosquito species rarely find conditions as suitable for breeding in the more natural countryside. This fact, together with the sparser human populations in the country, may account for the relative infrequency of truly rural cases. Sylvan transmission to wild birds and fowl is possibly accomplished by a widespread nondomestic species such as *C. salinarius*, which has been shown to be an excellent transmitter in the laboratory.[11]

The mechanics of an urban epidemic appear clear. Infected wild birds probably introduce the virus to high populations of suburban and urban *C. pipiens* or *C. quinquefasciatus*, which rapidly spread it to city dwelling birds and chickens. These in turn serve as a ready source of infection for additional numbers of the mosquitoes. A consequence of the resultant epizootic is exposure of a large segment of the human population to infection by mosquito bite.

A perplexing feature of the epidemiology of SLE is the importance of the *C. quinquefasciatus pipiens* complex in the East in the absence of *C. tarsalis* but its relative unimportance in its presence in the Far West. Obviously the balance of ecological factors that determine vector efficiency is tipped in its favor in the East but against it in the West. Although many contributing factors may be involved, the most likely appear to be strain differences in either the mosquitoes or the virus in the respective areas.

Studies comparing the transmitting efficiencies of *C. tarsalis* and 7 strains of *C. pipiens* and *C. quinquefasciatus* from various parts of the country showed that all transmitted a California strain of SLE virus from chick to chick with nearly 100 per cent efficiency.[11, 13] The threshold of infection of *C. tarsalis* appeared to be the lowest, however, with virus in such low concentration as to be undetectable in 1:10 diluted blood adequate for infection.[1] That this finding may be significant is suggested by comparing the results of studies on the viremias produced in birds by the California virus strain[39] and a strain from Kentucky.[8] The Kentucky strain of SLE virus was found to develop about a tenfold greater concentration in the blood.

In a discussion of this rather flimsy but provocative evidence with W. C. Reeves of the School of Public Health, University of California, Berkeley, Calif., a hypothesis was developed to account for SLE vector differences in

California and the East If *C tarsalis* is abundant and more susceptible than the other mosquito species there may be little natural selection for high bird viremia virus strains As a consequence the virus strains might characteristically produce viremias in birds too low for efficient infection of California *C quinquefasciatus* In the East however in the absence of *C tarsalis* it is possible that the only virus strains propagated would be those that produced viremias in birds adequate to infect *C quinquefasciatus pipiens* or other natural vectors of similar susceptibility with the resultant establishment of relatively high bird viremia strains in that area

In spite of a considerable knowledge of vector relationships control measures through vector abatement are often impracticable from an economic point of view Only urban SLE gives immediate promise of relatively inexpensive control It is obvious therefore that the reasons for continued investigations on the ecology of encephalitis are not merely academic There is a definite need to gain additional information that might lead to more effective control Phases particularly needing study are the overwintering mechanisms the finer points of vector host relationships and the influence of climatic and immunological factors upon disease spread If these phases were fully understood outbreaks might be predictable and control more surely and economically effected

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vector in that area and is responsible for most of the transmission to wild birds domestic fowl and man Up to 1953 69 isolations of the virus had been made from wild caught *C tarsalis* as against only 3 for *C quinquefasciatus* 2 for *C stigmatosoma* and 1 for *A dorsalis*[3] In 1954 alone 87 additional isolations from *C tarsalis* were reported[17] The greatest outbreaks have coincided with an abundance of *C tarsalis* and a high proportion of the cases have been rural rather than urban[40] As might be expected the epidemiology is similar in many respects to that of WEE

A different epidemiological picture has been presented in the central and east central states namely in the outbreaks in St Louis Mo 1933 Hidalgo County, Texas 1954 Calvert City Ky 1955 and Louisville Ky 1956 In those areas *C tarsalis* was scarce or absent There the human cases were mostly urban or suburban and they were associated with heavy breeding of *C pipiens* or *C quinquefasciatus*[5, 6, 20, 25, 41] These mosquito species rarely find conditions as suitable for breeding in the more natural countryside This fact, together with the sparser human populations in the country may account for the relative infrequency of truly rural cases Sylvan transmission to wild birds and fowl is possibly accomplished by a widespread nondomestic species such as *C salinarius* which has been shown to be an excellent transmitter in the laboratory[11]

The mechanics of an urban epidemic appear clear Infected wild birds probably introduce the virus to high populations of suburban and urban *C pipiens* or *C quinquefasciatus* which rapidly spread it to city dwelling birds and chickens These in turn serve as a ready source of infection for additional numbers of the mosquitoes A consequence of the resultant epizootic is exposure of a large segment of the human population to infection by mosquito bite

A perplexing feature of the epidemiology of SLE is the importance of the *C quinquefasciatus pipiens* complex in the East in the absence of *C tarsalis* but its relative unimportance in its presence in the Far West Obviously the balance of ecological factors that determine vector efficiency is tipped in its favor in the East but against it in the West Although many contributing factors may be involved the most likely appear to be strain differences in either the mosquitoes or the virus in the respective areas

Studies comparing the transmitting efficiencies of *C tarsalis* and 7 strains of *C pipiens* and *C quinquefasciatus* from various parts of the country showed that all transmitted a California strain of SLE virus from chick to chick with nearly 100 per cent efficiency[11, 13] The threshold of infection of *C tarsalis* appeared to be the lowest however with virus in such low concentration as to be undetectable in 1 10 diluted blood adequate for infection[34] That this finding may be significant is suggested by comparing the results of studies on the viremias produced in birds by the California virus strain[10] and a strain from Kentucky[8] The Kentucky strain of SLE virus was found to develop about a tenfold greater concentration in the blood

In a discussion of this rather flimsy but provocative evidence with W C Reeves of the School of Public Health University of California Berkeley Calif a hypothesis was developed to account for SLE vector differences in

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Stamm has demonstrated that a 4 week old CFW strain of laboratory mice has a demonstrable viremia for 3 days following subcutaneous inoculation with titers ranging from 10^{3} to 10^{-7} LD$_{50}$ However these latter rodents represent an artificial situation having been bred and selected for special susceptibility to infection with the neurotropic viruses

Birds have been shown to circulate fairly small amounts of SLE virus after experimental infection Hammon Reeves and Sather[3] showed that the virus could be detected in the blood of house finches English sparrows tricolored red winged blackbirds and white crowned sparrows In all except the white crowned sparrows virus was demonstrated only in the undiluted blood or rarely in the 10^{-1} dilution The white crowned sparrows however showed viremia titers as high as $10^{3.8}$ and $10^{4.4}$ LD$_{50}$ The antibody response was negligible during the first 2 months after inoculation Chamberlain *et al*[4] followed the response of individual birds infected by both subcutaneous inoculation and by the bite of infected mosquitoes Cowbirds red winged blackbirds English sparrows and the domestic pigeon were the species included in the study The pigeons and a portion of the cowbirds and red wings were tested by inoculating a 10^{-1} dilution of the blood into mice Of 15 birds so tested none of the subcutaneously inoculated cowbirds or red wings showed a demonstrable viremia in this diluted blood One pigeon showed traces of virus in the 10^{-1} dilution of blood on the fourth and fifth days following infection and the other on the seventh day One of 3 red wings infected by mosquito bite showed a trace of virus in diluted blood on the first day as did a second individual on the seventh day One of 2 cowbirds similarly infected showed a titer of $10^{1.6}$ LD$_{50}$ on the fourth day A second series of birds was inoculated subcutaneously and the blood tested as undiluted material in mice for detection of virus Three red wings circulated virus intermittently for 5 to 8 days following inoculation usually insufficient virus was present to kill all mice inoculated The highest titer observed in the red wings was $10^{1.5}$ LD$_{50}$ found in one bird on the sixth day Of 4 cowbirds similarly tested 2 failed to have any demonstrable virus in the undiluted blood for 14 days subsequent to inoculation One cowbird had a trace of virus in the undiluted blood through 5 days after inoculation while the fourth bird had a maximum titer of $10^{2.1}$ LD$_{50}$ on the sixth day One of 8 English sparrows also tested by inoculation of undiluted blood into mice failed to show a demonstrable viremia during the first 7 days after inoculation One sparrow had a trace of virus in its blood on the seventh day 1 a trace on the fifth day and another had traces of blood virus on the first 2 days after inoculation The remaining 4 sparrows had maximum virus titers of $10^{1.3}$ LD$_{50}$ to 10^{-5} LD$_{50}$ occurring on the fourth and fifth days after inoculation These 4 birds had viremic periods lasting for from 4 to 6 days Antibody response in all except the pigeons was either absent or very weak when tested at 25 to 30 days after inoculation

All of the previously mentioned experimental infections of birds with SLE virus were done using viruses isolated in California A strain of SLE virus isolated from a naturally infected bird from Kentucky produced somewhat higher viremia levels in cowbirds[5] Three birds inoculated subcutaneously had viremia for 7 days following inoculation Two of these had titers above

HOST RELATIONSHIP OF THE ARTHROPOD BORNE ENCEPHALITIDES

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One of the distinguishing characteristics of the arthropod borne viruses is that of a fairly wide host range By definition these viruses are capable of infecting both invertebrates and vertebrates Among the vertebrates many species of mammals and birds may be infected with these viruses However each virus tends to have one class that will furnish the more important hosts

The importance of a vertebrate as a host for the encephalitis viruses depends upon several factors First the individual must circulate virus in its blood in sufficient concentration and for a reasonable length of time to allow the invertebrate vector to become infected by ingesting the virus containing blood meal Moreover the vertebrate host to be of consequence in the continuing life cycle of the virus must choose a habitat also suitable for the invertebrate vector and by its habits allow sufficient opportunity for the arthropod to obtain blood meals

When the principal vectors are determined many otherwise potential vertebrate hosts can be dismissed because their preferred habitat does not coincide with that of the vector In the case of eastern equine encephalitis (EEE) in which the principal endemic vector appears to be *Culiseta melanura* (a swamp-breeding mosquito) certain upland game birds burrowing rodents or any other vertebrate preferring a relatively dry habitat could not enter into the endemic cycle of the virus Also since *C melanura* is preferentially a bird feeder mammals such as otters and swamp rabbits even when they choose a swamp habitat are not likely to become involved in the cycle of EEE infection In fact serologic studies on small mammals resident in swamps endemic for EEE have failed to show any evidence of experience with the virus

Although serologic surveys indicate which individuals are exposed to the vector and which are capable of being infected by the virus this information does not tell us which species can serve as hosts capable of passing the virus to additional vectors

St Louis encephalitis (SLE) virus is found at very low levels in the blood of any species during the viremic phase of infection This is compensated however by the fact that the arthropod vectors are able to become infected on blood meals containing very small amounts of virus

Cox Philip and Kilpatrick[1] found no virus circulating in the blood of 3 horses inoculated intracerebrally with SLE virus Stamm found that only 1 of 4 cottontail rabbits circulated SLE virus after subcutaneous inoculation This 1 animal had a minimal amount of virus present in its blood during a 3 day period after inoculation only 1 of 5 mice died when inoculated with the 1 2 dilution of blood Three of the 4 rabbits developed neutralizing antibody Six roof rats (*Rattus rattus*) were also inoculated subcutaneously with SLE virus None showed demonstrable virus in a 10^{-1} dilution of blood during the 4 days after inoculation nor did antibody develop in any of the rats

In some areas of the country chickens have a high rate of natural antibody to WEE while in other areas where the virus is also present negligible numbers possess antibody

In the case of WEE virus it again appears that birds are the primary hosts of the virus *wild birds being especially incriminated* *Domestic poultry may* play a secondary role depending upon the vectors accessibility to them

Eastern equine encephalitis (EEE) virus has a somewhat wider host range among mammals than either SLE or WEE viruses Birds appear to be uniformly susceptible

Horses have been found to circulate EEE[11,13] virus in their blood after experimental infection The virus appears with a fair degree of regularity but its presence is of short duration usually for 1 to 3 days only The maximum virus titers observed have ranged from $10^{0.5}$ to $10^{6.2}$ LD_{50} Titers in the range of $10^{3.0}$ LD_{50} are the maximum usually observed Horse to-horse transmission through the bite of mosquitoes has been shown experimentally[12,14] but in at least one case the infecting horse displayed an unusually high titer of blood virus at the time of the mosquito feeding

Davis[15] *has shown that mourning doves red winged blackbirds grackles* cowbirds pigeons and English sparrows have a viremia period following inoculation with EEE virus Kissling and his co workers[16-18] added the white ibis American egret snowy egret cardinal and pheasant to the list of birds susceptible by subcutaneous inoculation Stamm[19] has found that the yellow crown night heron white throated sparrow blue jay tufted titmouse meadow lark song sparrow towhee hermit thrush and brown thrasher are also susceptible In short no bird so far tested that is free of antibody to the virus of EEE has failed to respond to inoculation of virus with a period of viremia

The usual period of viremia in birds infected with EEE virus is 4 days The maximum blood virus titers obtained vary with the species of bird Large birds such as the herons and pheasants will have titers reaching from $10^{7.0}$ to $10^{5.0}$ LD_{50} The smaller birds such as grackles cardinals and sparrows will have maximum viremia titers from $10^{6.0}$ to $10^{9.0}$ LD_{50}

Domestic poultry have fairly low viremia titers and serologic surveys of chickens in endemic and epidemic areas have shown only an occasional individual with antibody to EEE indicating very little association with the vectors of the virus

Serologic surveys have limited the natural endemic hosts of EEE to birds more especially wild birds Of the wild birds the smaller species are more efficient hosts than are the larger birds as shown by the height of viremia that develops during infection Also small birds are more numerous and occur in greater concentration Horses may occasionally enter into the epidemic maintenance of EEE and since they are closely associated with humans they could assume some importance as an indirect source of human infection

Venezuelan equine encephalitis (VEE) virus is one of the arthropod borne viruses that seem to multiply with greater efficiency in mammals than in birds

Horses infected experimentally with VEE virus circulate virus for 4 to 6 days[20] Maximum blood virus titers were found to range from $10^{4.0}$ to 10^{5}

$10^{1.0}$ LD_{50}. Fourteen English sparrows also were inoculated with the Kentucky virus, using a small dosage. These birds were tested for viremia on the third day after inoculation and then at 6- to 7-day intervals through the twenty-third day. At 3 days 8 of the birds were viremic. At 9 days 4 of these were still viremic and an additional previously negative bird had viremia at this time. At 16 and 23 days 1 bird still had viremia; when this bird died on the twenty-seventh day, SLE virus was isolated from the spleen and kidneys but not from the brain or liver.

Chickens have been observed to develop a high rate of antibody to SLE virus during the course of an epidemic. Experimental studies on chickens show that the viremia levels of adults are comparable to those found in wild birds; exceptionally high titers are observed in young birds.

Thus, even with the limited information available we are able to narrow the list of important vertebrate hosts of SLE to birds, with special emphasis on the smaller perching birds such as sparrows and finches. Domestic poultry in areas where the principal vector of SLE is *Culex pipiens quinquefasciatus* are also severely suspect as important epidemic hosts, since they are a preferred host for the vector.

Western equine encephalitis (WEE) virus appears to multiply to higher levels in the blood of birds than does SLE virus and, in addition, it causes clinical disease in more species of mammals than does SLE virus.

It has been found difficult to produce clinical disease of WEE in horses by subcutaneous inoculation of virus.[6] The viremia levels in horses so inoculated are very low and fleeting. Usually virus is not present in sufficient quantities to infect mice by intracerebral inoculation, and it has been necessary to resort to the inoculation of one-half-day-old chicks[7] to demonstrate the traces of virus which are present in the blood of horses during the viremic phase.

Other mammals have not been investigated as far as WEE viremia levels are concerned.

Using a California strain of WEE virus, Hammon *et al.*[2] found that viremia was present in English sparrows, house finches, tricolored red-winged blackbirds, and white-crowned sparrows following subcutaneous inoculation of virus. Although individual birds were not followed daily, virus was recovered from some individuals as late as 9 and 10 days after inoculation. Most recoveries of virus were made during the first 4 days after inoculation. The maximum titers of $10^{4.0}$ to $10^{6.0}$ LD_{50} were found during the first 2 days after inoculation. Kissling *et al.*[8] followed the daily viremia patterns of individual birds including towhees, cardinals, English sparrows, white-throated sparrows, blue jays, and purple finches after subcutaneous inoculation with a WEE virus isolated from Louisiana. Viremia was detected in each species with maximum virus titers comparable to those observed by Hammon and his co-workers.[2] The periods of demonstrable viremia ranged from 2 to 6 days, the majority of the birds being viremic for 4 days. Kaplan, Winn, and Palmer[9] found that the common pigeon has blood virus levels and a viremic period similar to those of the other species of birds tested. Domestic fowl have been shown to circulate sufficient virus in their blood to allow infection of *Culex tarsalis*.[10] The blood virus levels in WEE-infected chickens are generally lower than those found in wild birds

In some areas of the country chickens have a high rate of natural antibody to WEF while in other areas where the virus is also present negligible numbers possess antibody

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LD_{50} These high titers were maintained for longer periods of time than were the maximum blood virus titers in horses infected with EEE virus No diffi culty was experienced in infecting certain species of mosquitoes by allowing them to feed upon horses in the viremic stage

Very little has been done with other species of mammals but it has been shown that the usual laboratory mammals are quite susceptible to peripheral inoculation of virus and at least in guinea pigs viremia titers approximate those found in horses

Wild birds especially those native to North America when infected with VEE virus do not have viremia levels as high or of as long duration as horses[21] In fact occasional individuals especially doves and pigeons seem to be re fractory to infection Birds in the viremic phase of the disease seldom had sufficient blood virus present to allow mosquito infection VEE viremia periods in these birds rarely exceeded 3 days

The United States probably has little to fear concerning VEE since it is apparently dependent upon a mammal reservoir Such a disease would have difficulty in becoming permanently established in areas where the mammalian wildlife has been reduced to the low point now prevailing in the United States Also due to the restricted movement of mammals as compared with that of birds such a disease would probably remain very limited geographically should it be introduced

In summary the information now available seems to indicate that mammals do not circulate enough virus in their blood during the viremic phase of the disease to be of any importance as a source of virus for epidemic arthropod vectors of SLE or WEE viruses Horses may occasionally provide an infective blood meal for mosquitoes during the viremic phase of EEE virus infection However the blood of horses infected with VEE virus is an excellent source of virus for mosquitoes Birds are excellent sources of virus for mosquitoes when infected with SLE WEE and EEE viruses but are rather poor hosts in this respect during the viremic phase of VEE infection

Although many or perhaps all species of birds are capable of circulating the encephalitis viruses in quantities sufficient for the infection of mosquitoes other factors must be considered in assaying the relative importance of any particular species These factors are those characteristics or habits that allow ex posure to the virus Thus it has been observed that gregarious species such as white ibis and yellow crowned night herons show a high rate of antibody to EEE virus[6,18] In addition to other factors such as longevity which could account for this observation the heavy concentration of young susceptible birds in a small area during the nesting season no doubt offers an optimum op portunity for the virus to become disseminated The gregariousness of a species must however be associated with the selection of a suitable habitat since it has been observed that gulls or even herons nesting on coastal islands show every little or no antibody to EEE Although these latter birds are exposed to many mosquitoes the species are those that may serve as epidemic vectors rather than those species found in endemic situations If the virus were introduced under these conditions such birds could add an important

impetus to the spread of an epidemic but they are of little or no importance in the long term survival of the virus

The selection of a particular type of habitat is also of importance in smaller species During endemic times isolations of EEE virus have been confined to such birds as thrushes catbirds and grackles species preferring moist swampy woodland Under epidemic conditions isolations of EEE have been made from English sparrows pigeons wild pheasants and other such birds species that are normally found in more open areas Surveillance for infection in these latter species could probably furnish warning for an incipient epidemic in humans or horses

Whether or not these viruses may acquire properties that favor epidemic spread or whether the occurrence of an epidemic is dependent solely upon the favorable coincidence of virus heavy mosquito populations and a dense susceptible bird population is not definitely known It is interesting however that a strain of EEE virus isolated under an endemic condition produced inapparent infections and maximum viremia titers of $10^{2.8}$ to $10^{5.5}$ LD_{50} in pheasants while a strain isolated from pheasants during an epidemic period produced maximum viremia titers in pheasants of $10^{4.3}$ to $10^{[illegible]}$ LD_{50} accompanied by clinical disease Likewise a strain of SLE virus isolated from a bird during an epidemic in Kentucky produces higher viremia titers in birds than does a California strain isolated during an endemic period

In the experimental infections of birds observed at the Montgomery laboratory virus has never been recovered from birds later than two weeks after the initial infection except in two instances both of which were SLE infections One would expect that a second viremia would be of a fleeting nature cut short by a rapid antibody recall and that as such it would offer very limited opportunities for the infection of mosquitoes However mosquito transmission may not be the only method of transfer of virus from bird to bird since transmission of EEE occurs with ease among captive pheasants even in the absence of arthropod vectors The poor antibody response in birds following SLE infection should encourage the exploration of the latency problem in this particular disease In the cases of EEE and WEE however it has been our experience that antibody response in birds is rapid following the termination of viremia and persists for long periods

Working on the hypothesis that the latent virus may be masked in some form a limited experiment was carried out in which the tissues of sparrows which had recovered from SLE infections were grown in tissue cultures In this manner it would be possible to free the tissues of antibody and the rapid proliferation of tissue might encourage a build up of virus These experiments met with negative results

However the question of latency in the vertebrate host cannot be dismissed since many possible mechanisms have yet to be explored

Thus far the vertebrate hosts have been neglected in the application of control measures for the encephalitides yet in the absence of a suitable host population the virus could not exist

Due to the type of habitat in which EEE virus is found it is unlikely that

mosquito abatement could ever be practicable for the control of this disease It is only under unusual circumstances that a human infection or even a horse infection would be expected to result from an exposure to mosquitoes feeding on swamp inhabiting birds The birds that live in proximity to human habitations are the more likely sources for virus that infects man and his domestic animals Therefore an effort to reduce the populations of certain pest birds such as English sparrows, starlings, and pigeons would be in order whenever this disease threatens

Much more must be known concerning the identity of the endemic hosts for SLE virus However, the severity of epidemics of SLE in urban areas of the central United States might be lessened by population control of the less desirable birds that serve as epidemic hosts of the virus

For any of the arthropod borne encephalitides, a combined attack on both the vectors and epidemic hosts should reduce the activity of the virus to a level below that rendered potentially possible by efforts directed toward only one member of the infection cycle More information is needed concerning the bird population concentration that is needed to support epizootic activity of these viruses

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EQUINE ENCEPHALITIS VACCINE STUDIES IN PHEASANTS UNDER EPIZOOTIC AND PRE EPIZOOTIC CONDITIONS

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Eastern equine encephalitis (EEE) was first reported in ring necked pheasants by Tyzzer Sellards and Bennett in Connecticut in 1938 [1] Beaudette *et al* described 27 outbreaks from 1939 to 1953 occurring in pheasant flocks raised commercially in New Jersey [8] Beaudette concluded EEE occurs with sufficient frequency to pose a serious economic problem to breeders of ring necked pheasants in New Jersey and perhaps in other highly endemic areas For this reason and because of its public health importance further effort should be made to elucidate the epidemiology of EEE and to devise practical methods for its control [8]

Prior field trials with respect to the efficacy of an EEE vaccine in pheasants were made by Holden[9] and by Beaudette [8] Holden stated with respect to a planned vaccine study in 1953 Results of the immunization procedure and the experimental manipulations of the birds were not conclusive A November storm destroyed the outside rearing pens releasing most of the birds therein Therefore, it was impossible to determine relative attack rates in immunized and control birds Beaudette stated The outcome in nonvaccinated and vaccinated at Forked River in 1946 was rather spectacular in that the disease did not appear in any vaccinated pen In previous studies however Beaudette *et al* [8] had noted that Proportions in excess of ninety per cent of the population in an individual pen may show signs of disease Other birds of the same age in contiguous pens may escape disease for weeks or perhaps altogether It is therefore possible that birds in the pen in which all were vaccinated were never under viral challenge In the same year Beaudette[6] reported an outbreak among vaccinated birds on the New Gretna N J farm investigated No actual mortality figures were available

Under controlled laboratory conditions Kissling[10] observed a survival rate of 67 per cent (6/9) in a group of pheasants that were vaccinated with a single

dose of formolized EEE vaccine after challenge with a strain of virus that caused a 70 per cent (7/10) mortality in nonvaccinated pheasants This apparent protection occurred in spite of the fact that no demonstrable neutralizing antibody appeared following use of a single dose of formolized vaccine

The work discussed in this paper is an attempt under controlled field conditions to evaluate the usefulness of a formolized chick embryo vaccine in the control of naturally occurring EEE within individual pens of commercially raised pheasants The study was made during the 1956 epizootic in New Jersey

Materials and Methods

In August 1956 a commercial pheasant breeding farm at Deans N J with approximately 5000 pheasants had lost more than 1500 birds The virus of EEE had been isolated by F R Beaudette of Rutgers University New Brunswick N J from some of these birds FIGURE 1 illustrates the distribution of pens at the farm

The disease first appeared August 16 in Pen 3 which contained 1800 birds 11 weeks of age (hatched May 28 1956) By September 4 the epidemic in this pen had subsided after a mortality of 48 9 per cent The disease next appeared August 22 in Pen 1 (A and B) which contained 1800 birds 13 weeks of age (hatched May 21 1956) In FIGURE 1 it can be seen that Section A of Pen 1 and Pen 3 are contiguous for a distance of 150 feet All pens were constructed of chicken wire were 7 feet high and had supporting posts 12 feet apart A ceiling of chicken wire or twine netting supported by posts 12

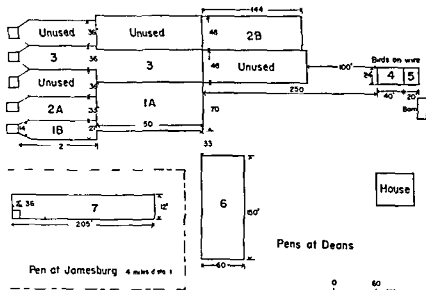


FIGURE 1 Diagram of the layouts of the pheasant farms at Deans and Jamesburg N J

TABLE 1

COMPARISON OF MORTALITY RATES OF PHEASANTS DURING THE FIRST SEVEN DAYS OF AN EPIDEMIC OF EEE IN PEN 1 AND PEN 7, SUMMER OF 1956

Location	First seven days of outbreak of EEE				
	Living	Dead	Total	Per cent mortality	Standard error of per cent
Pen 1	1563	237	1800	13.17	±0.79
Pen 7	524	74	598	12.37	±1.37
Total	2087	311	2398	12.97	±0.69

Critical ratio = 0.4995; X^2 = 0.2495. Interpretation: no difference.

feet apart prevented the birds from escaping. At the time of our initial visit on August 27, 1956, 238 birds in Pen 1 (A and B) had died.

Adjacent to Pen 1 (A and B) was Pen 2A, which contained 860 pheasants (hatched June 26, 1956) showing no signs of encephalitis on August 27, 1956. The pheasants in Pens 1, 2, and 3 were raised on the ground.

Pens 4 and 5 were on platforms 4 feet from the ground. The birds kept in them were thus said to be raised "on wire" and were never in contact with the ground. Pen 4 contained 400 birds (hatched July 4, 1956) and Pen 5 contained 100 birds (hatched May 7, 1956). Pen 6 (on the ground) contained 600 pheasants retained as breeders from the previous year (1955). Pen 4 was 250 feet from both Pen 1A and Pen 3. Pen 6 was 33 feet from Pen 1A.

On September 19 the disease broke out at a farm near Jamesburg four miles distant from the farm at Deans and separated from it by a swamp (Pigeon Swamp). Both the Deans and Jamesburg establishments were located on sandy soil typical of the coastal plain of New Jersey. The Jamesburg farm obtained its pheasants (hatched May 28, 1956) as day-old chicks from the Deans farm. The Jamesburg pen will be referred to as Pen 7 and is included in the vaccination studies. Statistical analysis of the Jamesburg outbreak (TABLE 1) establishes the comparability of the mortality rate of Pen 7 and the mortality rate in Pen 1 (A and B) at Deans.

The vaccine was utilized under two conditions: (1) in affected Pens 1 (A and B) and 7 seven days after outbreak, and (2) in pre-epidemic Pen 2A and nonaffected Pen 4. Each bird received 0.2 ml. of undiluted formolized chick embryo bivalent vaccine* injected intramuscularly into the pectoral region. A repeating type vaccinating syringe was used.† One needle (1¼ in., 20 gauge) was used for 50 to 75 birds. In Pens 1A and 2A birds were handled in alternating groups of 10 to 15. The vaccinates were stained with an alcoholic methyl green solution. The nonvaccinated controls were stained with an alcoholic carbol fuchsin solution, in both instances on the medial surface of the left wing. The nonvaccinated pheasants were allowed to run with the vaccinated birds in the same area. Birds failing to respond to drives in Pen

* This vaccine was generously provided by Samuel Bozeman of Pitman-Moore Laboratories, Indianapolis, Ind.

† Vapol Intramuscular Vaccinator, produced by Vineland Poultry Laboratories, Vineland, N. J.

1A were classified as 'nonvaccinated—not handled' In Pen 4 vaccine was injected into all the birds Pen 5 was maintained as a nonvaccinated control for the birds raised on wire

Dead birds were picked up daily and the pens in which they died their vaccination status and the date of death were recorded All heads were frozen for virus isolation Birds were opened and observed for macroscopic (post mortem) lesions of diseases other than encephalitis Blood samples were taken from representative birds in all pens at varying intervals

The procedure employed was to vaccinate the pheasants in the unaffected pens before entering those in which the epidemic was present The birds first vaccinated were the 400 raised on wire in Pen 4 those in Pen 5 were left unvaccinated as its control The next group handled comprised the birds in Pen 2A 530 (61 per cent) of the 860 total number of birds were vaccinated by August 28 On September 19 (21 days later) the breeder moved 402 of the best feathered least picked birds to Pen 2B Of this group 243 (60 4 per cent) had been vaccinated Pen 2B had not been used the previous season and the birds in it were debeaked prior to placing them in the new pen On September 21 (23 days after vaccination) the disease broke out in Pen 2A of the 458 birds that had been left in this pen 280 (61 1 per cent) had been vaccinated

Before vaccinating in Pen 1 where an epidemic prevailed the pen was divided into Sections 1A and 1B Prior to this division there was feed and water in both sections of the pen and the birds passed freely from one part to the other No attempt was made to count or to select birds for either section The separation was made by erecting a chicken wire fence from ground to roof netting Four hundred fifty (450) birds were left in Pen 1B to serve as nonvaccinated controls for Pen 1A In Pen 1A there were 1113 pheasants Birds were driven in successive drives into a catching enclosure Of this handled group 540 were vaccinated and marked with green dye 288 were not vaccinated and were marked with red dye The remaining 285 birds that did not respond to the drives were noted to be in poorer condition than the handled birds These nonvaccinated birds were subsequently referred to as not handled—no color in the mortality records In Pen 1A as a whole 48 5 per cent of the 1113 birds were vaccinated on August 29 and 30

Pen 7 at Jamesburg containing 598 pheasants experienced an outbreak of EEE on September 19 The diagnosis was confirmed by virus isolation at Rutgers University by Beaudette On September 26 the remaining 524 birds in Pen 7 were vaccinated

There were thus 4 groups of pens involved

Group 1 representing 3 proportions of vaccinated to nonvaccinated pheasants 7 days after outbreaks of the disease in the pen all raised on the ground (A) Pen 1B 0 0 per cent vaccinated (B) Pen 1A 48 5 per cent vaccinated and (C) Pen 7 100 0 per cent vaccinated

Group 2 consisting of 2 groups of birds vaccinated when no EEE disease was present (A) Pen 2A 61 1 per cent vaccinated (birds raised on the ground) (B) Pen 2B 60 4 per cent vaccinated (birds raised on the ground) and (C) Pen 4 100 0 per cent vaccinated (birds raised on the wire)

Group 3 included 2 pens of nonvaccinated and healthy birds (A) Pen 5 birds on wire and (B) Pen 6 breeders (on the ground)

Group 4 the first pen to be affected in which no attempt had been made to curb the disease (A) Pen 3

Results

Pens vaccinated 7 days after outbreak The daily mortality in Pens 1A 1B and 7 following vaccination is given in TABLE 2 The results are graphically illustrated in FIGURE 2 which shows the survival curves of pheasants plotted according to the actuarial method employed in life tables as described by Berkson and Gage[11] The survival curves of the vaccinated groups level off about the ninth day after vaccination whereas that of the nonvaccinated birds in Pen 1B continued its downward descent to the thirteenth day The differences in the three are statistically significant (TABLE 3)

FIGURE 3 shows the survival curves within Pen 1A where 48 5 per cent of the 1113 birds available were vaccinated As previously indicated the non

TABLE 2

TABULATION OF DEATHS AMONG BIRDS IN PENS VACCINATED SEVEN DAYS AFTER OUTBREAK

No days after vaccination	Pen 1A					Pen 1B 100% All dead birds	Pen 7 100% vacc All dead birds
	51 5% nonvaccinated			48 5% Vaccinated	Total 1A All dead birds		
	Handled dead	Not handled	Total				
1	8	24	32	10	42	26	11
2	12	14	26	12	38	12	9
3	20	30	50	29	79	30	45
4	23	19	42	26	68	23	18
5	33	37	70	32	102	30	15
6	11	39	50	11	61	26	25
7	6	22	28	7	35	24	16
8	12	22	34	11	45	36	5
9	2	4	6	—	6	38	2
10	—	3	3	2	5	41	3
11	—	5	5	—	5	12	3
12	—	1	1	—	1	10	—
13	—	1	1	—	1	3	2
14	—	1	1	—	1	3	—
15	—	1	1	—	1	2	—
16	—	—	—	—	—	—	1
17	—	—	—	—	—	—	1
20	—	1	1	1	2	—	—
22	2	—	2	—	2	1	—
24	—	—	—	—	—	3	—
Number of dead birds	129	224	353	141	494	320	156
Number of birds at time of vaccination	288	285	573	540	1113	450	524

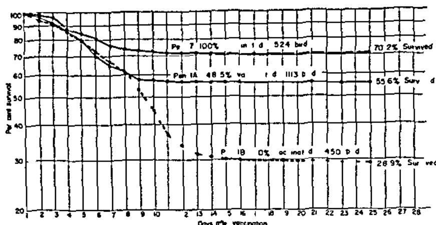


FIGURE 2 Survival curves by pens of pheasants vaccinated seven days after the outbreak of the epizootic

TABLE 3
NUMBER AND PER CENT OF PHEASANTS LIVING OR DEAD VACCINATED SEVEN DAYS AFTER OUTBREAK OF FEF SUMMER 1956

Location	Number			Per cent	
	Living	Dead	Total	Mortality	SE
Pen 7 100% vaccinated	368	156	524	29 8	±2 00
Pen 1A 0 0% vaccinated	619	494	1113	44 4	±1 49
Pen 1B 48 5% vaccinated	130	320	450	71 1	±2 14

X^2 = 170 Interpretation reliable difference

vaccinates that were not handled were in poorer physical condition and their survival rate was low (21 4 per cent) Of the handled birds the vaccinated portion had the highest survival rate (73 9 per cent) whereas the nonvaccinated handled portion had a lower survival rate (55 2 per cent) a statistically significant difference (TABLE 4) The nonvaccinated total group represents both the handled and nonhandled birds

FIGURE 4 presents survival curves of vaccinated and nonvaccinated portions of the birds in Pen 1A as compared to those of the totally vaccinated birds in Pen 7 and the totally nonvaccinated occupants of Pen 1B The survival rates of those comprising the vaccinated portion of Pen 1A and the 100 0 per cent vaccinated Pen 7 were similar and TABLE 5 indicates that no statistical difference exists between these two rates The survival rates for the pheasants in the nonvaccinated portion of Pen 1A and the entire population of Pen 1B in which no birds were vaccinated were statistically different (TABLE 6) It appears that the benefits of the vaccine do not confine themselves merely to birds vaccinated a more favorable survival rate is found among susceptible birds remaining in the pen In a disease spread by bird to bird contact the

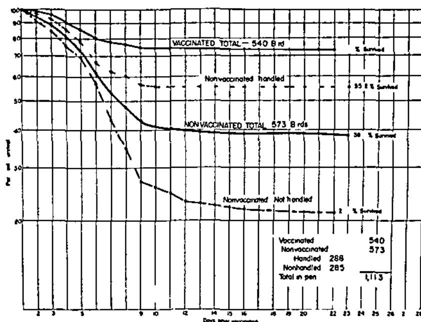


FIGURE 3 Survival curves of pheasants in Pen 1A vaccinated seven days after the outbreak of the epizootic

TABLE 4

COMPARISON OF MORTALITY RATES OF VACCINATED AND NONVACCINATED *HANDLED* BIRDS PEN 1A

Pheasants in Pen 1A	Living	Dead	Total	Per cent mortality	Standard error
Vaccinated green	399	141	540	26 1	±1 9
Nonvaccinated red	159	129	288	44 8	±2 9
Total	558	270	878	32 6	±1 6

Critical ratio = 5 2 X^2 = 27 5 Interpretation reliable difference

greater the number of protected birds the more difficult it becomes for the virus to reach the decreased number of susceptibles In birds vaccinated during this epidemic results indicate that regardless of the proportion of individuals vaccinated the survival rates in the vaccinated birds remained fairly constant

FIGURE 5 indicates the per cent occurrence of deaths of pheasants in Pens 1B 1A and 7 It will be noted that the peak occurrence of mortality occurred on the third day following vaccination in the 100 per cent vaccinated group (Pen 7) on the fifth day following vaccination in the 48 5 per cent vaccinated group (Pen 1A) and on the tenth day following vaccination in the 0 0 per cent vaccinated group (Pen 1B) Vaccination in the midst of this epidemic shortened the time necessary to reach the peak of epidemic loss and decreased the number of individuals that succumbed

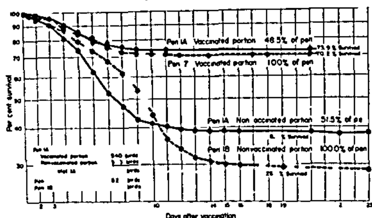


FIGURE 4 Survival curves of vaccinated and nonvaccinated birds of Pen 1A as compared to those of totally vaccinated birds of Pen 7 and of the totally non accinated birds of Pen 1B

TABLE 5
COMPARISON OF MORTALITY RATES OF VACCINATED PHEASANTS IN PENS 1A AND 7

	Living	Dead	Total	Per cent mortality	Standard error
Vaccinated Pen 1A	399	141	540	26 1	±1 9
Vaccinated Pen 7	368	156	524	29 8	±2 0
Total	767	297	1064	27 9	±1 4

Critical ratio = 1 33 X^2 = 1 77 Interpretation no difference

TABLE 6
COMPARISON OF MORTALITY RATES OF NONVACCINATED PHEASANTS IN PENS 1A AND 1B

	Living	Dead	Total	Per cent mortality	Standard error
Nonvaccinated Pen 1A	220	353	573	61 6	±2 0
Nonvaccinated Pen 1B	130	320	450	71 1	±2 1
Total	350	673	1023	65 8	±1 5

Critical ratio = 3 2 X^2 = 10 1 Interpretation different

TABLE 7 and FIGURE 6 summarize the results in this group of birds vaccinated seven days after an outbreak occurred

Pen vaccinated 23 days prior to outbreak TABLE 8 presents the daily mortality in Pens 2A and 2B The outbreak first appeared in Pen 2A on September 21 23 days after 61 1 per cent of the birds had been vaccinated The birds in Pen 2B had been moved from 2A on September 19 2 days prior to epidemic onset in Pen 2A

FIGURE 7 indicates the survival curves for Pen 2A The significant dif

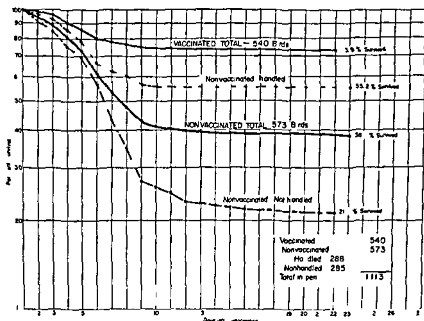


FIGURE 3 Survival curves of pheasants in Pen 1A vaccinated seven days after the outbreak of the epizootic

TABLE 4
COMPARISON OF MORTALITY RATES OF VACCINATED AND NONVACCINATED HANDLED BIRDS PEN 1A

Pheasant Pen 1A	Living	Dead	Total	Per cent mortality	Standard error of per cent
Vaccinated green	399	141	540	26 1	±1 9
Nonvaccinated red	159	129	288	44 8	±2 9
Total	558	270	828	32 6	±1 6

Critical ratio = 5 2 χ^2 = 27 5 Interpretation reliable difference

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TABLE 7

NUMBER AND PER CENT OF PHEASANTS LIVING AND DEAD VACCINATED SEVEN DAYS AFTER OUTBREAK OF EEE SUMMER 19[illegible]

Locat	Number			Per cent			
	Living	Dead	Total	Survival	Mortality	Standard error of rate	Vaccinated
Pen 7	368	156	524	0.2	29.8	±2.0	100.0
Pen 1B	130	320	450	28.9	71.1	±2.1	0.0
Pen 1A	619	494	1113	55.6	44.4	±1.5	48.5
Vaccinated green	399	141	540	73.9	26.1	±1.9	100.0
Nonvaccinated	220	353	573	38.4	61.6	±2.0	0.0
Handled red	159	129	288	55.2	44.8	±2.9	0.0
Not handled no color	61	224	285	21.4	78.6	±2.4	0.0

TABLE 8

TABULATION OF DEATHS AMONG BIRDS VACCINATED 23 DAYS PRIOR TO OUTBREAK

Day of outbreak	Pen 2A			Pen 2B
	Nonvaccinates	Vaccinates	All dead birds	All dead birds
1	4	1	5	
2	4	—	4	
3	3	—	3	1 (nonvaccinate)
4	4	1	5	
5	11	—	11	2 (nonvaccinates)
6	10	2	12	
7	10	—	10	
8	17	4	21	
9	11	1	12	
10	11	4	15	
11	11	5	16	
12	8	4	12	
13	4	6	10	
14	5	3	8	
15	3	—	3	
16	1	3	4	
17	2	—	2	
18	3	1	4	
19	1	—	1	
20	—	—	—	
21	1	—	1	
22	—	—	—	
Number of dead birds	124	35	159	3
Number of birds	178	280	458	402

FIGURE 9 summarizes the findings in Pen 2A. Of the 178 nonvaccinated birds 124 or 69.7 per cent succumbed whereas of the 280 vaccinated only 36 or 12.5 per cent died.

In Pen 2B three birds, all nonvaccinated, died from the disease. Since

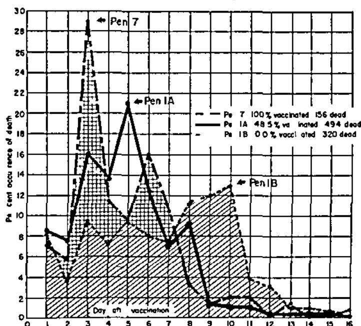


FIGURE 5 Per cent occurrence of deaths in pens in which the birds were vaccinated seven days after the outbreak of the epizootic

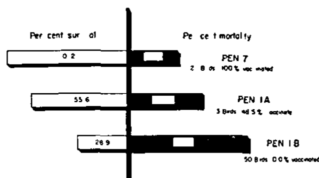


FIGURE 6 Survival and mortality rates of birds vaccinated seven days after the outbreak of the epizootic

ference in survival rates between the vaccinated (87 5 per cent) and the non vaccinated portions (30 3 per cent) are readily observable FIGURE 8 illustrates the percentage of deaths occurring in Pen 2A The epidemic ran its normal 21 day course and the peak mortality rate occurred on the eighth day of the epidemic for the nonvaccinated group and on the thirteenth day for the vaccinates Of the vaccinated group the birds succumbing resisted the challenge for a longer interval of time This would suggest that a vaccine break occurred only after a sufficient virus challenge had been built up within the pen

TABLE 7

NUMBER AND PER CENT OF PHEASANTS LIVING AND DEAD VACCINATED SEVEN DAYS AFTER OUTBREAK OF EEE SUMMER 1956

Location	Number			Per cent			
	Living	Dead	Total	Survival	Mortality	Standard error of per cent	Vaccinated
Pen 7	368	156	5 4	70 2	29 8	±2 0	100 0
Pen 1B	130	320	450	28 9	71 1	±2 1	0 0
Pen 1A	619	494	1113	55 6	44 4	±1 5	48 5
Vaccinated green	399	141	540	73 9	26 1	±1 9	100 0
Nonvaccinated	220	353	573	38 4	61 6	±2 0	0 0
Handled red	159	129	288	55 2	44 8	±2 9	0 0
Not handled no color	61	224	285	21 4	78 6	±2 4	0 0

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2	4	—	4	
3	3	—	3	1 (nonvaccinate)
4	4	1	5	
5	11	—	11	2 (nonvaccinates)
6	10	2	12	
7	10	—	10	
8	17	4	21	
9	11	1	12	
10	11	4	15	
11	11	5	16	
12	8	4	12	
13	4	6	10	
14	5	3	8	
15	3	—	3	
16	1	3	4	
17	2	—	2	
18	3	1	4	
19	1	—	1	
20	—	—	—	
21	1	—	1	
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Number of dead birds	124	35	159	3
Number of birds	178	280	458	402

FIGURE 9 summarizes the findings in Pen 2A Of the 178 nonvaccinated birds 124 or 69 7 per cent succumbed whereas of the 280 vaccinated only 36 or 12 5 per cent died

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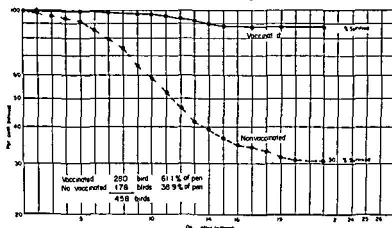


FIGURE 7 Survival curves of birds in Pen 2A vaccinated twenty three days prior to the outbreak of the epizootic

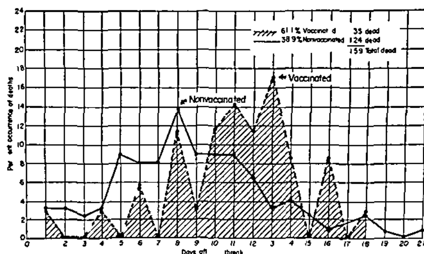


FIGURE 8 Per cent occurrence of deaths of birds in Pen 2A vaccinated twenty three days prior to the outbreak of the epizootic

these birds were moved two days before the onset of the epidemic in Pen 2A it is possible that they succumbed to an infection contracted in Pen 2A prior to their transfer It is difficult to ascertain whether the failure of the disease to reach epidemic proportions in the new Pen 2B should be attributed to (1) the debeaking performed as the birds were transferred (2) the fact that they were placed in a new clean pen or (3) the better condition of the birds at the time they were selected

No losses due to the disease were noted in Pens 4 5 or 6 Sera obtained from Pens 5 and 6 indicate negative results in these disease free pens of non vaccinated birds TABLE 9 summarizes the results for these pens and for Pens 2A and 2B

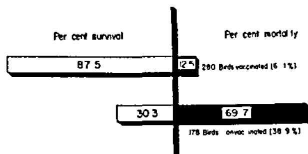


FIGURE 9 Survival and mortality rates of birds in Pen 2A vaccinated twenty three days prior to the outbreak of the epizootic

TABLE 9
NUMBER AND PER CENT OF PHEASANTS LIVING AND DEAD IN PENS UNAFFECTED AT TIME OF VACCINATION SUMMER 1956

Location	Number			Per cent			
	Living	Dead	Total	Survival	Mortality	Standard error of per cent	Vaccinated
Pen 2A	299	159	458	65.3	34.7	2.22	61.1
Vaccinated	245	35	280	87.5	12.5	1.98	100.0
Nonvaccinated	54	124	178	30.3	69.7	3.45	0.0
Pen 2B	399	3	402	99.3	0.7	0.42	60.4
Vaccinated	243	0	243	100.0	0.0	—	100.0
Nonvaccinated	156	3	159	98.1	1.9	1.08	0.0
Pen 4	400	0	400	100.0	0.0	—	100.0
Pen 5	100	0	100	100.0	0.0	—	0.0
Pen 6	600	0	600	100.0	0.0	—	0.0

It is apparent from the results in Pen 2A that vaccination prior to outbreak did in fact offer substantial protection (87.5 per cent survival) to those birds vaccinated. Only under the severe challenge produced by allowing susceptible controls to remain within the pen did some single vaccinated birds break (12.5 per cent). The authors feel that if 100 per cent of the birds in the pen had been vaccinated prior to exposure the viral build up would have been limited and the vaccine breaks would have occurred less frequently if at all. The question of debeaking versus vaccination must be settled by wildlife management personnel and commercial breeders on the basis of ability of the birds to survive in nature upon release and by the economics of the industry.

Summary

The efficacy of vaccine in an epidemic and pre epidemic situation of EEE was evaluated in New Jersey during the 1956 epizootic. The vaccine was proved effective under the conditions of the experiment.

Under controlled conditions pens of pheasants that had undergone the first week of the 3 week disease cycle were vaccinated. The resulting mortality

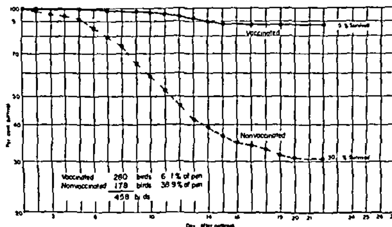


FIGURE 7 Survival curves of birds in Pen 2A vaccinated twenty three days prior to the outbreak of the epizootic

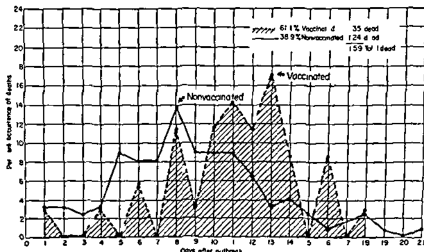


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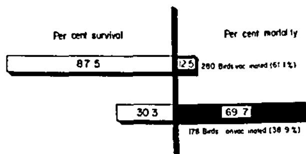


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NUMBER AND PER CENT OF PHEASANTS LIVING AND DEAD IN PENS UNAFFECTED AT TIME OF VACCINATION SUMMER 1956

Location	Number			Per cent			
	Living	Dead	Total	Survival	Mortality	Standard error of rate	Vaccinated
Pen 2A	299	159	458	65.3	34.7	2.22	61.1
Vaccinated	245	35	280	87.5	12.5	1.98	100.0
Nonvaccinated	54	124	178	30.3	69.7	3.45	0.0
Pen 2B	399	3	402	99.3	0.7	0.42	60.4
Vaccinated	243	0	243	100.0	0.0	—	100.0
Nonvaccinated	156	3	159	98.1	1.9	1.08	0.0
Pen 4	400	0	400	100.0	0.0	—	100.0
Pen 5	100	0	100	100.0	0.0	—	0.0
Pen 6	600	0	600	100.0	0.0	—	0.0

It is apparent from the results in Pen 2A that vaccination prior to outbreak did in fact offer substantial protection (87.5 per cent survival) to those birds vaccinated Only under the severe challenge produced by allowing susceptible controls to remain within the pen did some single vaccinated birds break (12.5 per cent) The authors feel that if 100 per cent of the birds in the pen had been vaccinated prior to exposure the viral build up would have been limited and the vaccine "breaks" would have occurred less frequently if at all The question of debeaking versus vaccination must be settled by wildlife management personnel and commercial breeders on the basis of ability of the birds to survive in nature upon release and by the economics of the industry

Summary

The efficacy of vaccine in an epidemic and pre epidemic situation of EEE was evaluated in New Jersey during the 1956 epizootic The vaccine was proved effective under the conditions of the experiment

Under controlled conditions pens of pheasants that had undergone the first week of the 3 week disease cycle were vaccinated The resulting mortality

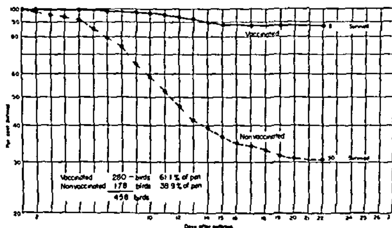


FIGURE 7 Survival curves of birds in Pen 2A vaccinated twenty three days prior to the outbreak of the epizootic

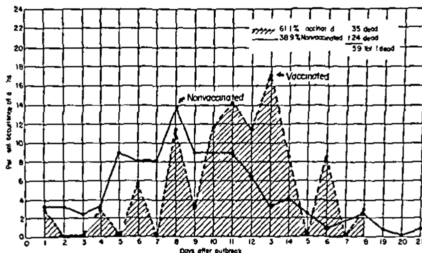


FIGURE 8 Per cent occurrence of deaths of birds in Pen 2A vaccinated twenty three days prior to the outbreak of the epizootic

these birds were moved two days before the onset of the epidemic in Pen 2A it is possible that they succumbed to an infection contracted in Pen 2A prior to their transfer It is difficult to ascertain whether the failure of the disease to reach epidemic proportions in the new Pen 2B should be attributed to (1) the debeaking performed as the birds were transferred (2) the fact that they were placed in a new clean pen or (3) the better condition of the birds at the time they were selected

No losses due to the disease were noted in Pens 4 5 or 6 Sera obtained from Pens 5 and 6 indicate negative results in these disease free pens of non vaccinated birds TABLE 9 summarizes the results for these pens and for Pens 2A and 2B

Discussion of the Paper

S A Goldberg (*Presbyterian Hospital Newark N J*) While examining the late D H Udall s collection of slides from an enzootic disease of horses in Kansas thought to be caused by a certain toxin from a plant in the pastures *it was noted that the principal lesion in the brain was perivascular lymphocytic* infiltration mantling which is similar to the lesion in encephalitis

Because lymphocytes are extremely radiosensitive it was thought that radiotherapy should be useful in the treatment of acute encephalitis

To verify this hypothesis mice were inoculated intranasally with the St Louis strain of encephalitis virus Treatment consisted of one fourth erythema dose started one day after inoculation

Of 38 mice treated the incubation period varied from 6 to 11 days Nineteen survived Of the others 1 survived 6 days 4 survived 8 days and 14 survived from 9 to 11 days

Of 53 mice similarly inoculated but not treated the period of incubation varied between 5 and 8 days All but 1 lived only 1 day after symptoms appeared Only 1 survived

Of 7 human cases treated all recovered and none developed postencephalitic Parkinson s syndrome after 10 years of observation

Portman and Lough tested this treatment on 49 cases of acute encephalitis Of these 59 per cent were considered recovered and 30 per cent improved

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rates were as follows 29 8 per cent (100 per cent vaccinated) 44 4 per cent (48 5 per cent vaccinated) 71 1 per cent (0 per cent vaccinated) Analysis of the results within the 48 5 per cent pens of vaccinated birds indicates that the vaccine protected the vaccinated birds and to a degree the nonvaccinated birds as well The use of vaccine influenced the epidemic curve so that the peak of mortality occurred earlier in pens of vaccinated birds as opposed to the pens in which the birds were nonvaccinated

The utilization of a vaccine on 61 1 per cent of the birds in a pen 23 days prior to an outbreak indicated that the vaccine produced sufficient immunity in that period to protect 87 5 per cent of the birds vaccinated as opposed to a survival rate of 30 3 per cent of the nonvaccinated controls A study of daily mortality indicates that the peak mortality in the vaccinates occurred in the second week of the 3 week epidemic as opposed to a first week peak noted in the susceptible controls This suggests that a vaccine break" occurred only after a sufficient virus challenge had been built up within the pen

Acknowledgments

The authors gratefully acknowledge the assistance rendered this project by William C Carter and Robert F Goldsboro of the New Jersey State Department of Health Milton C Clark and Olive and Evelyn Oberman owners of the Deans farm Foster Abele owner of the Jamesburg farm A Heaton Underhill and Lester C MacNamara of the New Jersey Division of Fish and Game Fred R Beaudette (deceased) and David C Tudor Viral Laboratory of Rutgers University Raymond Fagan of the Wyeth Research Laboratory Richard E Shope of The Rockefeller Institute for Medical Research New York N Y and Marguerite F Hall Carl E Weigele and Elmer L Shaffer all of the New Jersey State Department of Health

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ORPHAN VIRUSES OF MAN AND ANIMALS*

By G D Hsiung and Joseph L Melnick†
Yale University School of Medicine New Haven Conn

INTRODUCTION

The increased use of tissue culture methods in the field of virology has resulted in the discovery of many cytopathogenic agents These viral agents may be recovered from the tissues and excreta of man and the lower animals many of them defy detection by methods other than cell cultivation Although we have excellent methods for the detection of these newly recognized viruses we are only beginning to learn about the diseases if any that they may produce These agents have been called 'orphan viruses because we know so little about the diseases with which they may be associated As so many of them have been isolated from the intestinal tract they have been considered with the enteroviruses It is well known that families of viruses such as those of herpes and the pox group exist in different species and now it appears that we also have enteroviruses for a number of different animal species such as enteric cytopathogenic human orphan virus (ECHO) enteric cytopathogenic monkey orphan virus (ECMO) enteric cytopathogenic bovine orphan virus (ECBO) and enteric cytopathogenic swine orphan virus (ECSO)

ECHO Viruses[1]

The human enteric viruses to which the orphan viruses belong are not like those enteric bacteria that inhabit the intestinal tract for the life of the individual The proportion of persons carrying enteric viruses decreases as age increases Observations to date indicate that the enteric viruses produce only transitory infections the seriousness of which depends upon the type and strain of virus

Although some ECHO viruses have been recovered from cases of infant diarrhea and from patients with aseptic meningitis the epidemiological criteria regarding all of the ECHO viruses as etiological agents of these syndromes have not yet been met However certain members of this group have been associated with outbreaks of aseptic meningitis[2-4] and certain types notably 5 6 and 9 and perhaps others have been isolated from the cerebrospinal fluid In Europe in 1956 ECHO Type 9 accounted for thousands of cases of aseptic meningitis often with rash[6,7] and in 1957 the epidemic spread into the United States Other ECHO viruses Types 4 and 16 have also been associated with cases of rash as well as with aseptic meningitis The epidemic strains of ECHO Type 9 prevalent in Europe in 1955 and 1956 seem to be antigenic variants of the prototype strain[4]

In addition to the association of the currently prevalent ECHO Type 9

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strains with outbreaks of aseptic meningitis one other important fact has come out of these studies namely that certain of these virulent strains after passage in monkey kidney tissue produce paralysis and myositis in infant mice Mice become paralyzed when inoculated by subcutaneous or other routes and histologically exhibit a myositis similar to that found in animals inoculated with Group A Coxsackie viruses In view of these mouse pathogenic variants the question must be raised at this time (although it cannot be answered from the available evidence) whether ECHO Type 9 should be classified with the ECHO viruses or with the Coxsackie viruses

If a scheme of classification rests on the host range of the virus then the host range of the natural virus before laboratory manipulation might well be the criterion of choice This procedure certainly would make the test for host range easier to carry out than one that required as a preliminary to the test an indefinite number of tissue culture passages This leads to another problem in that we have isolated a number of Coxsackie A9 and Coxsackie B strains that fail to infect infant mice unless these agents are first passaged in tissue culture Obviously we are only at the beginning in the study of these agents which ultimately may well be grouped together for purposes of classification as enteroviruses

New Developments in Tissue Culture Methods for Studying Viruses of Man and Animals

Morphologic Characteristics of Plaques

The growth of animal viruses in cells under agar produces areas of necrosis resulting in virus colonies or plaques Differences in plaque patterns produced by various animal viruses have been noted by many investigators for instance Dulbecco with Newcastle virus and western equine encephalitis virus[8] Sellers with vesicular stomatitis and foot and mouth disease[9] and Dubes with different strains of poliovirus[10] Our own previous studies on the enteroviruses had shown[11, 12] that poliovirus Types I II and III produce large circular plaques with clear centers and sharp boundaries within 4 or 5 days after seeding on rhesus monkey (*Macaca mulatta*) kidney cultures (FIGURE 1) Coxsackie virus plaques (FIGURE 2) were also round resembling those of the polioviruses except for their delayed appearance which usually occurs 6 or 7 days after seeding Apparently healthy cells stained with neutral red could be seen within the area of degeneration this was especially marked at the periphery of the plaques of Coxsackie A9 In order to demonstrate the slow growing plaques of ECHO viruses it was necessary to adapt Dulbecco s technique[8] to stoppered bottle cultures[11] in which monkey kidney cells are stable for 14 days or longer under agar As shown in FIGURE 3a ECHO virus plaques are more irregular in shape than poliovirus plaques and their boundaries are diffuse exceptions are Types 7 and 12 (FIGURES 3b and 5a) which form plaques that are almost impossible to distinguish from those of the polioviruses Measles is another virus that was found to produce typical plaques in bottle cultures as shown in FIGURE 4 Minute plaques with sharp boundaries appeared as early as 4 days after inoculation into bottle cultures of *patas* monkey (*Erythrocebus patas*) kidney cells and enlarged although slowly thereafter



FIGURE 1 Plaque morphology of polioviruses 8 days after seeding of rhesus cultures poliovirus Type I (Mahoney) poliovirus Type II (MEF1) and poliovirus Type III (Saukett)

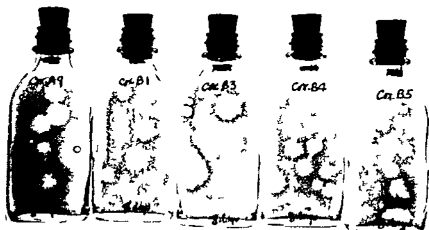


FIGURE 2 Plaque morphology of Coxsackie viruses 8 days after seeding of rhesus cultures Coxsackie viruses A9 (Craig) B1 (Connecticut—5) B3 (Nancy) B4 (Texas—130) and B5 (Faulkner)

Host Cell Susceptibility

Important in the characterization of a virus is the determination of its host range Thus it has been the general experience that adenoviruses may be more readily isolated in HeLa cells than in monkey kidney cultures while

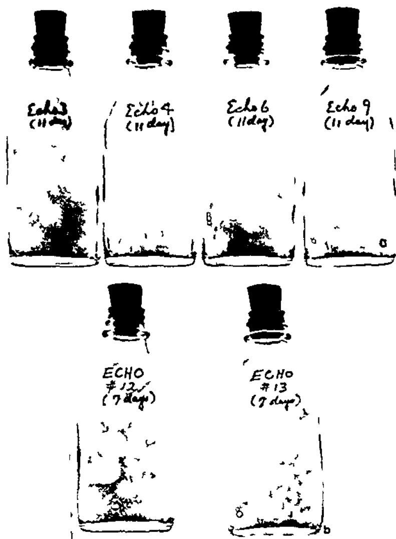


FIGURE 3 (a) Plaque morphology of representatives of Group A ECHO viruses on rhesus cultures 11 days after seeding Type 3 (Berard) Type 4 (Pesacek) Type 6 (D Meo) and Type 9 (Quigley) (b) Plaque morphology of representatives of the 2 groups of ECHO viruses on rhesus cultures 7 days after seeding right Group A ECHO Type 13 (Hamphill) left Group B ECHO Type 12 (Tra s)

the opposite is true for the ECHO viruses A comparative study on the susceptibility of kidney cells from different monkey species to poliovirus Coxsackie and ECHO viruses using the plaque method has been reported in detail[13] Of the species investigated only the kidney cells of rhesus and

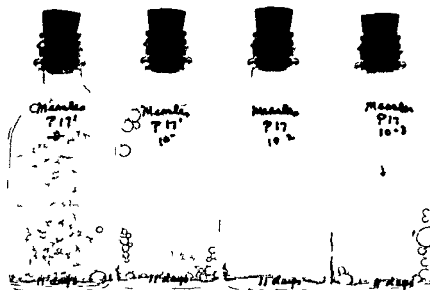


FIGURE 4 Plaques of measles virus (17th *patas* passage) in *patas* bottle cultures 11 days after seeding Dilutions undiluted tissue culture fluid 10^{-1} 10^{-2} and 10^{-3}

TABLE 1
COMPARATIVE SUSCEPTIBILITY OF RHESUS AND *PATAS* MONKEY KIDNEY CULTURES TO DIFFERENT VIRUSES

Virus	C nt t lt fl d f t s d	A e ge mber f plaq es p bottle— 0 1 ml oc lum	
		Rh	*pat*
Poliovirus	10^{-5}	8 9	23 5
Coxsackie A9	10^{-}	18 5	0
Coxsackie B1	10^{-}	17 0	33 5
ECHO Type 1	10^{-} 0	24 2	0
FCHO Type 7	10^{-} 0	25 3	?3 0
Measles	10^{-} 0	0	8 5

At the virus dilutions used in this experiment no plaques were formed however in cases where large virus inocula were used tiny delayed plaques occasionally appeared

patas the African red grass monkey will be considered here As shown in TABLE 1 *patas* cells were found to be twice as susceptible as rhesus cells to poliovirus and to Coxsackie B1 virus and about equal to rhesus cells in susceptibility to ECHO Type 7 In contrast Coxsackie A9 and ECHO Type 1 failed to form plaques in *patas* cultures Measles produced distinct plaques on *patas* monolayer but on rhesus culture it gave an irregular response at best

Isolation and Identification of ECHO Viruses

If we take advantage of differences in plaque morphology and cell susceptibility of rhesus and *patas* cultures ECHO viruses can be placed into two

TABLE 2

GROUPING OF ECHO VIRUSES

Group A (*patas* negative)
1 Small irregular plaques with hazy centers on rhesus only ECHO 3 (Berardi strain) 4 6 6 9 11 14 15 16 17 18 19
2 Small irregular plaques with clear centers on rhesus (occasionally tiny delayed plaques on *patas*) ECHO 1 13
3 No plaques on either rhesus or *patas* ECHO 2 3 5 6 10
Group B (*patas* positive)
1 Large circular plaques on rhesus and *patas* ECHO 7
2 Small or medium sized plaques on rhesus and tiny delayed plaques on *patas* ECHO 8
3 Large circular plaques on rhesus and tiny delayed plaques on *patas* ECHO 12

Except for the 6 prime strains and the Berardi strain of Type 3 all viruses listed are prototype strains

groups (TABLE 2) Group A contains ECHO virus Types 1 3 (Berardi strain) 4 6 9 11 13 14 15 16 17 18 and 19 these produce small irregularly shaped hazy plaques in rhesus cultures but are negative in *patas* cultures Plaques of ECHO Type 1 and ECHO Type 13 are clearer in the centers occasionally with large inocula of these types tiny delayed plaques appear on *patas* cultures Group B contains ECHO Types 7 8 and 12 these ECHO types are positive in *patas* cultures as well as in rhesus cultures ECHO Type 7 (FIGURE 5a) produces large circular plaques resembling those of poliovirus in both rhesus and *patas* cultures ECHO Type 8 (FIGURE 5b) produces smaller and more irregular plaques on rhesus cultures and tiny delayed plaques on *patas* cultures ECHO Type 12 produces large circular plaques on rhesus but small irregular plaques on *patas* cultures The prototype strains of ECHO Types 2 3 5 6 and 10 have not produced plaques in tube cultures they were found to be cytopathogenic for rhesus but not for *patas* cells and are considered to be members of Group A The reasons for the failure of these ECHO viruses to produce plaques are obscure particularly in the case of the Type 6 prototype strain (D Amori) which produces complete cytopathic changes in 48 hours when cells are kept in a fluid medium Although the cell sheet degenerated under agar when the inoculum of this strain was large no clear plaques have been observed

Difficulties have been encountered in identifying different strains of ECHO Type 8 isolated from various stool samples As shown in FIGURE 6a stool No 269 which was typed as ECHO Type 8 produced small irregular plaques on rhesus cells at 10^{-3} concentration and gave no plaques when an inoculum one thousandfold greater was placed on *patas* cultures Thus this strain of ECHO Type 8 is like those belonging to Group A On the other hand stool No 271 (FIGURE 6b) which was also typed serologically as ECHO Type 8 produced circular plaques on rhesus as well as on *patas* cells thus resembling ECHO Group B viruses A rhesus tissue culture passage of stool No 279 (FIGURE 6c) that produced plaques of medium size on rhesus cells but tiny delayed plaques on *patas* cells was grouped as ECHO Type 8 by its biological characteristics as well as by serologic typing

Presumptive grouping of stool isolates on the basis of plaque morphology

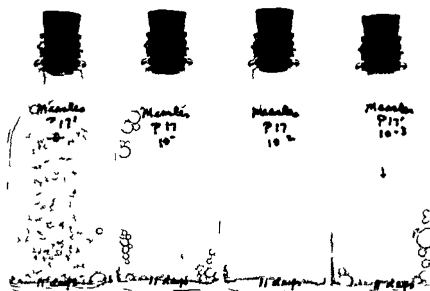


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		Rh	*p t s*
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Coxsackie A9	$10^{-6\ 7}$	18 5	0*
Coxsackie B1	10^{-4}	17 0	33 5
ECHO Type 1	10^{-}	24 2	0
ECHO Type 7	10^{-5}	25 3	23 0
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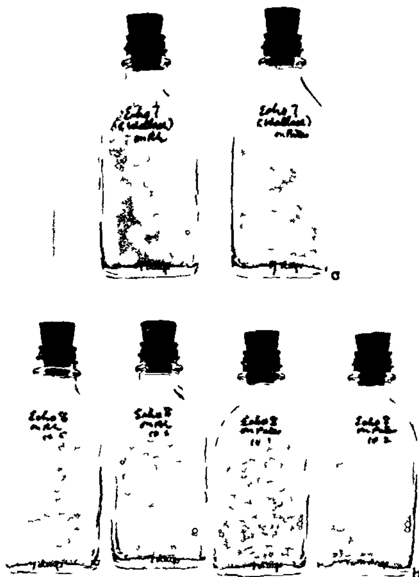


FIGURE 5 (a) Plaqu[es of] ECHO Type 7 (Wallace) on rhesus (left) and on patas (right) 7 days after seeding (b) Plaques of ECHO Type 8 (Bryson) on rhe[sus (2 bo]ttle[s] at left) and on *patas* (2 bottles at right) 7 days after seeding

and cell susceptibility followed by typing with specific antisera was carried out with a total of 70 enteric cytopathogenic agents that had been found to be negative for poliovirus and negative for Coxsackie virus in suckling mice The results are shown in TABLE 3 Of these 70 strains 6 were identified as Coxsackie viruses despite the fact that they had been negative in suckling mice

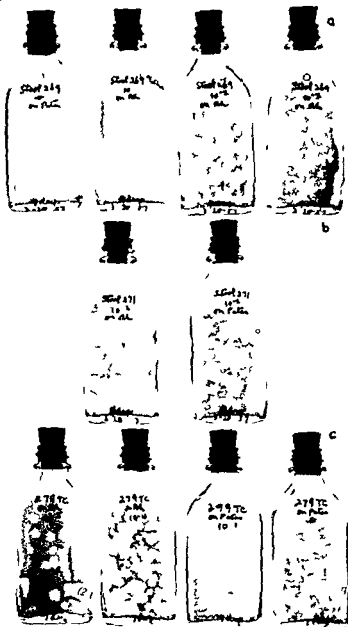


FIGURE 6 (a) Plaques of stool No 269 on rhesus (3 bottles at right dilutions 10^{-} 10^{-2} and 10^{-}) and on *patas* (bottle at left undiluted) 9 days after seeding (b) plaques of stool No 271 on rhesus (left) and on *patas* (right) 9 days after seeding (c) plaques of rhesus kidney passage from stool No 279 on rhesus (2 bottles at left dilutions 10^{-} and 10^{-6}) and on *patas* (2 bottles at right dilutions undiluted and 10^{-}) 7 days after seeding

TABLE 3
IDENTIFICATION OF ECHO VIRUSES

Source of sample	Number of tissue culture isolates*	Number typed as ECHO viruses†	Number typed as Coxsackie viruses	Number unidentified
Phoenix Ariz	34	26	0	8
Charleston W Va	25	20	2	3
Chicago Ill	11	7‡	4‡	1
Total	70	53‡	6‡	12

* Negative for poliovirus and negative in suckling mice
† Classified among Types 1–19
‡ One stool sample contained a mixture of Coxsackie Type B4 and ECHO Type 4

Fifty three strains fell into the 19 known ECHO types and only 12 have remained as unidentified agents Selection of the proper typing sera was made easier by the preliminary grouping based upon the above mentioned biological properties

Mixed Infections

Mixed infections were easily and quickly recognized by the use of the plaque technique FIGURE 7 shows 2 distinct kinds of plaques in a rhesus culture bottle inoculated with a stool suspension in *patas* cultures only large circular plaques were observed Because of the greater numbers of the large plaques on *patas* cultures it was assumed that the large circular plaques were those of



FIGURE 7 Plaques of mixed infection (stool No D11) on rhesus (left) and on *patas* (right) 7 days after seeding On the rhesus culture smaller ECHO plaques are visible in the lower portion of the bottle as well as the large circular poliovirus plaque in the upper portion Only poliovirus plaques appear on *patas*

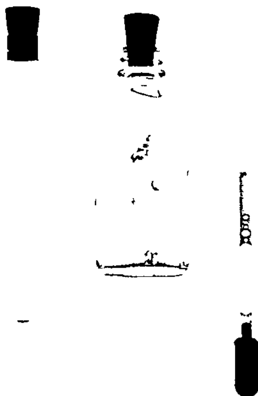


FIGURE 8 Technique for picking plaques center a bottle culture from which 2 kinds of plaques have been picked right capillary pipette that is used for picking left the tube culture in which material from the plaque is subcultured

polioviruses and because it failed to grow in *patas* cultures the virus producing the smaller irregular plaques on rhesus culture was tentatively classed as ECHO Group A Single plaques were picked as shown in FIGURE 8 using the capillary pipette and were passed into rhesus tube cultures in order to obtain a larger yield of virus for further identification In this case it was determined by use of specific antisera that poliovirus Type I and ECHO virus Type 1/ were present in this specimen Thus we see that by the plaque method 2 viruses were readily distinguished and a pure line of each virus could be established by passage and identified by typing with specific antisera

The problem of mixed infections is complicated when the ratio of poliovirus

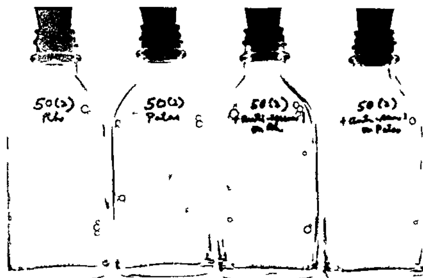


FIGURE 9 Mixed infection In the absence of poliovirus Type I antiserum poliovirus plaques of stool No 50(2) appeared on rhesus (left) and on *patas* (second from left) In the presence of this antiserum ECHO plaques appeared only on rhesus and not on *patas* (right)

to ECHO virus is excessive FIGURE 9 indicates such an example Stool No 50 grew on both rhesus and *patas* cultures However in the presence of polio Type I antiserum a certain amount of the virus failed to be neutralized These non neutralizable virus particles grew on rhesus but not on *patas* cultures Passage of plaques from the rhesus bottle containing poliovirus antiserum yielded a virus that was typed as ECHO Type 18

TABLE 4 illustrates some of the problems that a diagnostic laboratory encounters in regard to mixed enteric virus infections Six fecal specimens were tested by the conventional tube method in our laboratory (listed as Laboratory A) and three of these were also tested in a laboratory in another city (listed as Laboratory B) The first 3 specimens were typed in tube cultures as poliovirus Type I with the ECHO component which was present at low concentration being missed Poliovirus was missed from stool I C 6 presumably because in this instance the relative amount was very low The last 2 specimens listed in the table were identified in the tube test as mixtures of poliovirus and ECHO virus and here it is seen that the relative amounts of the viruses were more nearly equal Laboratory B missed the poliovirus component in the first 2 specimens listed presumably because the virus that it isolated could not be neutralized by polio antisera Stool II A 21(2) gave an interesting result A cytopathic agent was obtained after this sample was treated with poliovirus Type I antiserum Although the nonpolio agent thus obtained reacted with ECHO Type 4 antiserum it was also noted that it produced plaques on *patas* cultures Further study was therefore made and it was found that this specimen contained Coxsackie B4 and ECHO Type 4, in addition to poliovirus Type I

TABLE 4

COMPARISON OF METHODS FOR THE ISOLATION AND IDENTIFICATION OF ENTERIC VIRUSES IN MIXED INFECTIONS

Stool number	Tube method (CPE + neutralization)		Plaque method (Plaque morphology + host-cell susceptibility + neutralization)		
	Laboratory A	Laboratory B	Mixture	Per cent in mixture	
				Polio	ECHO
50(2)	Polio I	ECHO	Polio I + ECHO 18 (Group A)	87	13
II N 21(2)	Polio I	ECHO	Polio I + Coxs. B 4 + ECHO 4 (Group A)	84 (polio + Coxsackie)	16
5414	Polio I	Polio I + ECHO	Polio I + ECHO ? (Group A)†	99	1
I C 6	ECHO	N D	Polio I + ECHO 17 (Group A)	5	95
I D 11	Polio I + ECHO	N D	Polio I + ECHO 17 (Group A)	62	38
III B-40	Polio I + ECHO	N D	Polio I + ECHO 11 (Group A)	75	25

N D = not determined
† Not typed

It can be seen that by use of the plaque method and host-cell susceptibility mixed infections could be recognized more readily and the different agents could be separated into pure clones. However, if this method is used for initial virus isolation, rhesus tube cultures should be inoculated in parallel with bottle cultures in order to detect ECHO viruses that do not produce plaques.

ECMO Viruses *

Enteric cytopathogenic monkey orphan (ECMO) viruses are included among the simian viruses isolated from monkey kidney cultures by Hull and his colleagues[14-16]. These viruses have also been recovered from rectal swabs and fecal materials by Cheever[16] and by Riordan[17]. In one series of 21 monkeys studied in New Haven, Conn., 25 ECMO viruses were recovered. Some of these viruses were excreted for a period of weeks from the time of the first isolation. The high prevalence of these viruses in monkey stools has led to the question of whether viruses apparently recovered from the kidneys or from other tissues of the monkey are really viruses of these tissues, or whether they are ECMO viruses that had contaminated the tissue in the course of their removal from the body. If these viruses are as widespread as the present evidence suggests, then the skin and hair of monkeys must be heavily contaminated and contamination of the tissue must be considered as a possible source of these so-called kidney agents. A cooperative program with the laboratories of Hull and of Cheever is now under way in an attempt to classify

* Although foamy agents, as reported by Rustigian and Brown, are also simian viruses, they are not included in this discussion. It may be said, however, that in our studies we found that kidney cells of a number of African monkeys, in contrast to those from Asia, failed to contain foamy agents.

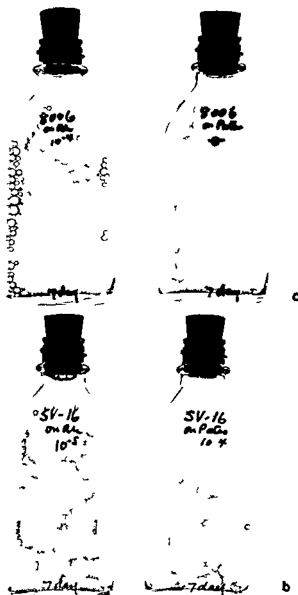


FIGURE 10 (a) Bottles inoculated with an ECMO virus of Group C (No 8006) Rhesus culture (left dilution 10) and *patas* culture (right undiluted) 7 days after seeding There are typical plaques with islets on rhesus but no plaques on *patas* (b) Bottles inoculated with ECMO Group B virus (No SV16) rhesus culture (left) and *patas* culture (right) 7 days after seeding Large circular plaques with islets are visible in cultures of both species

the numerous agents that have been isolated either from kidney cultures or from the stools of monkeys.

Biologic and Serologic Properties of Simian Orphan Viruses

A total of 51 strains of simian orphan viruses (isolated either from kidney cultures or from stool samples) were tested for their plaque morphology in rhesus bottle cultures and for their *patas* cell susceptibility.

Some simian viruses were found to produce plaques resembling those of the ECHO viruses described earlier; others have produced distinctive plaques characterized by the presence of islets of apparently healthy cells within the area of degeneration, as shown in FIGURE 10. *Ten strains were found to* produce distinct plaques with islets on rhesus cells and were negative in *patas* bottle cultures; these were placed in Group C (TABLE 5 and FIGURE 10a).

TABLE 5

PRESUMPTIVE GROUPING OF SIMIAN VIRUSES ACCORDING TO PLAQUE MORPHOLOGY AND *PATAS* CELL SUSCEPTIBILITY

Group A: small delayed plaques on rhesus cultures and on *patas* cultures			
7852	SV_1		P 4
7853	SV_4		P 5
7854	SV		P 6
7855A	SV		P 7
7999A	SV_6		P 8
	SV_7	SV_2	P 9
	SV_{23}	SV_7	P 10
	SV_2	SV_{28}	
Group B: large circular plaques with islets on rhesus cultures and on *patas* cultures			
7848	SV_2		P 1
7849	SV_6		P 3
7850	SV		P 11
7998			
8000			
8226†			
8228			
8229†			
8422			
8423			
8427			
8429			
Group C: large circular plaques with islets on rhesus cultures but *no plaques* on *patas* cultures			
8558	SV_6		P 2
7999B			
8006			
8227			
8425			
8426			
8431			
8432			

The strains listed in the column at the left were isolated in our laboratory; the SV series in the center column were made available by R. N. Hull, Lilly Research Laboratories, Indianapolis, Ind.; and the P series in the column at the right were supplied by F. S. Cheever, University of Pittsburgh, Pittsburgh, Pa.

† Mixed strains.



FIGURE 11. Bottles inoculated with a Group A ECMO virus (No. SV_1). Rhesus culture (2 at left) and *patas* culture (2 at right) 10 days after seeding. With the large inoculum (10^{-1}) generalized virus degeneration of the cell sheet occurred as soon as any plaques appeared. With the smaller inoculum on rhesus cultures as well as in the *patas* cultures, plaques were tiny and delayed.

Group B consisted of strains that produced distinct plaques with islets on both rhesus and *patas* cultures (FIGURE 10b). Group A included those that failed to produce plaques rapidly, although with large inocula the cell sheet degenerated under agar. However, tiny delayed plaques like those of measles virus usually occurred on *patas* as well as on rhesus cultures 10 days or more after inoculation (FIGURE 11). Some strains in the latter group gave positive complement fixation (CF) responses when allowed to react with human serum that previously had been shown to contain adenovirus antibodies.

Viruses 8226 (FIGURE 12) and 8229 were found to be mixtures. Purification of these virus mixtures was undertaken by picking single plaques, and further studies are under way.

Neutralization tests among the ECMO viruses may be carried out by the plaque method as shown in FIGURE 13. Type SV_6 of Hull and our strain No. 8227 were placed in Group C because of their plaque morphology and their failure to produce plaques on *patas* cultures. Neutralization of the No. 8227 virus by SV_6 antiserum indicated antigenic relationship. Similar correlations were found for SV_{16} and our strain No. 8000, which produced large clear plaques on both rhesus and *patas* (Group B) cultures, and for Type SV_3 and our strain 7854, which were placed in Group A because they were *patas* positive but produced only tiny delayed plaques. Thus with some of these viruses the plaque characteristics and host cell range could be correlated with antigenicity.

On the other hand, although some of the viruses were placed in different

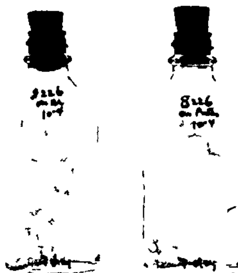


FIGURE 12 ECMO viruses (No 8226) on rhesus (left) and on patas (right) 7 days after seeding Two kinds of plaques were noted in rhesus bottle cultures some with and some without islets Only plaques without islets were observed on patas culture

groups according to their *patas* cell susceptibility (such as No 78551B which was *patas* negative and SV₂ which was *patas* positive) they were nevertheless found to be serologically related Cheever s 1 3 agent was a *patas* positive strain but it was neutralized by antiserum to SV₆ (a *patas* negative strain)

ECBO Viruses *

These viruses are agents isolated from the enteric tract of the bovine species From the fecal samples of healthy dairy cattle Kunin and Minuse [9] isolated 8 viral agents in tissue cultures of bovine kidney None of these agents was neutralized by polio Coxsackie or ECHO anti era Moll and Finlayson [1] reported recently on a cytopathogenic viral agent isolated from feces of cattle with fever M Klein (personal communication) and Klein and Earley [m] have isolated about 40 viruses all but one from the feces of apparently healthy cows One virus wa latent in calf kidney culture None of the 35 agents tested were neutralized to any significant degree by polio antisera although neutralizing antibodies to all 3 types of poliovirus were noted in bovine sera [23] All of these agents were found to grow readily in bovine kidney cultures and in addition some have been found to propagate in monkey kidney but not in HeLa cultures It is not unlikely that they may have a biological pattern in the bovine species similar to that of the ECHO viruses in man

* Enteric cytopathogenic swine orphan (ECSO) viruses were isolated from the stools of newborn swine by Moscovici in Italy These agents grew rapidly in monkey and swine kidney cells but HeLa cells failed to respond

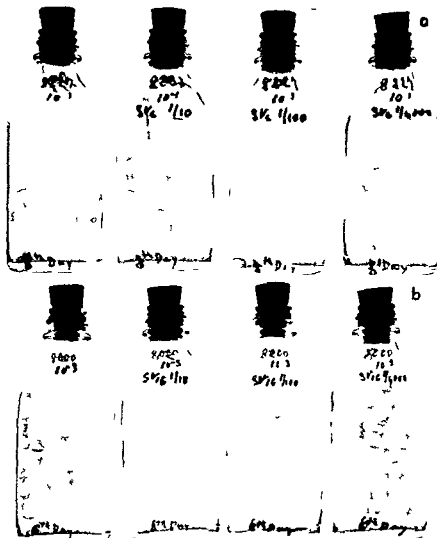


FIGURE 13 (a) Neutralization test in bottles with ECMO virus 8227 against SV antiserum in rhesus cultures 8 days after seeding. Virus control bottle at left contained 20 plaques. When SV antiserum was present at dilutions of 1:10 and 1:100 no plaques appeared; with an antiserum dilution of 1:1000 14 plaques were observed. (b) Neutralization test in bottles with ECMO virus No. 8000 against SV antiserum in rhesus cultures 6 days after seeding. Virus control bottle at left contained 42 plaques. No plaques appeared when SV antiserum was present at dilutions of 1:10 and 1:100; with an antiserum dilution of 1:1000 [illegible] plaques appeared.

That viral agents exist in the bovine species was already inferred from the fact that bovine sera neutralize certain viruses. For example, as mentioned earlier, naturally occurring substances that neutralize the three types of poliovirus have been found in normal adult bovine sera.[5] This phenomenon is discussed more fully elsewhere in these pages by Morton Klein. Sabin

and Fieldsteel[5] felt that these inhibitory substances are true antibodies which has led to the suggestion that some of the bovine orphan viruses may be related to polioviruses Recently Takemori and his colleagues[4] from Tokyo Japan have shown that one can distinguish between virus inhibitors in bovine sera and the true antibodies of poliovirus

By several passages of poliovirus Types I and II in HeLa cell cultures containing 20 to 40 per cent inhibitory normal bovine serum mutants were obtained that were able to produce large plaques in the presence of the inhibitory bovine serum in contrast to the small plaques of the parent virus[24] These mutants although resistant to the inhibitory normal bovine serum were no more resistant to true polio antibodies (as present in monkey immune serum) than were the parent viruses Therefore it may be concluded that the properties of the parent virus that were changed by passage in the inhibitory serum were not their specific polio antigenic characters Thus the inhibitory substances in normal bovine serum cannot necessarily be attributed to a response of the animal to infection with a poliovirus Only additional work can clarify the meaning of these viral inhibitory substances in bovine sera

Concluding Remarks

We have attempted to review some trends in research on orphan viruses New tissue culture methods continue to yield new viruses at an almost alarming rate from virtually every animal species studied

The orphan virus category was originally conceived as a place for the temporary classification of new agents until their diseases were found It may well be that with so many orphan viruses already recognized a number of them may turn out to infect man or lower animals without ever causing disease as is the case with saprophytic bacteria Other orphan viruses such as ECHO Types 6 and 9 cause aseptic meningitis As this syndrome is caused by a variety of viruses (including poliomyelitis Coxsackie and lymphocytic choriomeningitis) no single agent can be called *the* virus of aseptic meningitis This poses a difficult problem with reference to removing a virus from the orphan classification even when it is known to produce a disease if the same syndrome can be caused by other agents

For the next decade at least virologists will necessarily be concerned with working out methods for the recognition and classification of these agents We believe that colony or plaque morphology and cell susceptibility the traditional criteria for classification of bacteria are of similar value for classification of viruses Our recent studies with classifying 40 strains of ECHO and 51 strains of ECMO viruses may serve as an example We were able to group them provisionally by plaque morphology and host cell susceptibility without resorting to the tedious and time consuming serologic procedures required if serology alone had been used in relating them to the many antigenic types already known among the enteroviruses

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Discussion of the Paper

Henry M Gelfand (*School of Medicine Tulane University New Orleans La*) William Flynn and I at the Tulane University School of Medicine probably shall be able soon to add another group to the list of viruses presented by Hsiung and Melnick We believe that we may have isolated several members

of an ECDO group enteric cytopathogenic dog orphan viruses At least two strains have been obtained from a group of fecal specimens collected from apparently healthy stray dogs They were isolated in tissue cultures of dog kidney and seem to be adaptable to monkey kidney cells but not to HeLa cells Work has just begun with these agents but preliminary results indicate that they are not neutralized by rabbit hyperimmune sera against the 3 types of poliovirus against Coxsackie viruses A9 and B1 to B5 or against ECHO viruses Types 1 to 13 These ECDO viruses are neutralized by a majority of the dog sera against which they have been tested and also by a number of human sera They have not yet been tested for neutralization by specific sera against distemper virus or infectious canine hepatitis (ICH) virus but this will be done soon However it seems unlikely that these agents will prove to be either distemper or ICH virus Rabbits are being immunized for a more detailed antigenic study of relationships to human enteric agents

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Discussion of the Paper

HENRY M. GELFAND (*School of Medicine, Tulane University, New Orleans, La.*): William Flynn and I at the Tulane University School of Medicine probably shall be able soon to add another group to the list of viruses presented by Hsiung and Melnick. We believe that we may have isolated several members

TABLE 1

NEUTRALIZING ANTIBODIES IN COWS TO THREE TYPES OF POLIOVIRUS

Farm	No. tested	Per cent positive
A	81	43
B	32	70
C	18	72
D	25	68

Sera were tested at a dilution of 1:4 against 50 tissue culture doses of virus.

TABLE 2

INCIDENCE OF THREE TYPES OF NEUTRALIZING ANTIBODIES TO POLIOVIRUS IN COWS

	Per cent	
Type I	*Type II*	*Type III*
35	63	11

for our discussion the question of how one interprets neutralizing substances to certain human viruses in the sera of cows.[8,10] In our own studies we observed that there were present in the sera of several domestic animals, horses, hogs and particularly cows, neutralizing substances to Types I, II and III polioviruses. We have been following about 150 cows on 4 farms in the Philadelphia area; as shown in TABLE 1 the neutralizing substance to polioviruses is a widely distributed agent. As shown in TABLE 2 all 3 types of virus are neutralized. In addition we have found neutralizing substances to Types 4 and 5 Coxsackie B viruses and there have been recent reports of neutralizing substances to the adenoviruses in the sera of cows.[11] We may then ask this question: Are these neutralizing substances true antibodies that indicate an unusually wide distribution in animal sera of human viruses or their bovine antigenic relatives? The problem of interpreting these serologic reactions is rather awkward because we have no clinical picture to guide us, no epidemiological pattern and no acute and convalescent sera to detect a rise in antibody titer. We are confronted with individual sera from apparently healthy cows that neutralize these human viruses. When studied further these sera yielded the following information:

(1) The neutralizing substance is heat stable, resisting 60° C. for 30 min.

(2) The inhibitor is in the globulin fraction obtained by precipitation with ammonium sulphate, one third saturation.

(3) The inhibitor is type specific; some sera inhibit Type I, others Type II or Type III, still others combinations of all three or none at all.

(4) There is evidence of passive transfer, for newborn calves have neutralizing substances that disappear in a month or so and gradually reappear as the animal matures, which is the traditional antibody pattern.

(5) Neutralizing titers vary from 0 to greater than 1:250 against 50 tissue culture doses of virus, which is again a pattern consistent with an antibody interpretation.

Do the above data permit us to conclude that cows have antibodies to Types I, II and III polioviruses or do other substances exist in serum that mimic this antibody pattern?

THE SIGNIFICANCE OF HUMAN ANTIVIRAL NEUTRALIZING SUBSTANCES IN ANIMAL SERA*

By Morton Klein

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In considering infections that are common to man and animals probably the greatest unexplored area is that of the viruses Tissue culture has given virologists the golden touch and there are now pouring forth an enormous number of newly recognized human and animal viruses If one considers that about eighty infections[1] of animals are already known to be shared in common with man one can predict that a study of these newly isolated human and animal viruses will reveal many examples of identity or at least, of antigenic relationships

As we all know one of the most sensitive and specific tools for determining relationships between viruses is the antigen antibody reaction In virology we use three basic procedures the complement fixation test the inhibition of hemagglutination test and the neutralization test An essential requirement of all these tests is that they be specific for if one explores the sera of an animal species and finds something that neutralizes a test virus one would very much like to say the virus is or has been here Unfortunately serologic tests are never carried out as pure antigen antibody reactions they take place in the rather unpredictable menstruum of serum and there are many things in sera that can give grossly misleading serologic results It is the intent of this discussion to consider those substances that may disturb the specificity of these serologic reactions

The literature on unexplained inhibitors in animal sera is a collection of many miscellaneous scattered observations with no central theme discernible other than the fact that there is a variety of inhibitors One can consider these inhibitors from different points of view their role in nonspecific resistance to infection and the problems they raise when used as media in virus tissue culture work For the present discussion I have arbitrarily organized the material to try to answer this specific question How can one recognize a true antibody among a collection of unknown inhibitors?

I shall exclude from any detailed consideration a large number of nonantibody inhibitors that may be extracted from body cells One can obtain from phagocytic cells such microbial inhibitors as leukins[2] and phagocytin[3] one can extract from red blood cells an agent that inhibits viral hemagglutination[4] certain polypeptides obtained from tissues are known to be inhibitory[5] a mucopolysaccharide from the intestinal tract inhibits Theiler s virus[6] aqueous extracts from brain and spleen[7] are known inhibitors of microbial activity None of these cellular inhibitors need concern us here since as far as we know they do not appear in the sera of normal animals

We shall consider the problem of nonspecific inhibitors by using as the basis

The work reported in this paper was supported in part by a grant from the National Foundation for Infantile Paralysis Inc New York N Y

feres with the hemagglutination test of arthropod viruses[21] the particle is so large it can be removed by high speed centrifugation or slow filtration (3) in dengue there is a serum inhibitor of the hemagglutination test that must be removed with acetone[22] In the encephalomyocarditis group of viruses a nonspecific inhibitor of hemagglutination is frequently observed[23] These nonspecific inhibitors of hemagglutination need not be confused with true antibodies both for the reasons stated above and because they do not in general neutralize infectivity

As for the neutralization test I know of only one heat stable nonglobulin inhibitor of viral neutralization that is the serum lipids reported to give very low levels of neutralization against certain viruses[24, 5] It is well to note that highly abnormal sera might give nonspecific neutralization reactions For example sera obtained from jaundiced patients with yellow fever or infectious hepatitis may contain enough bile products to neutralize a virus in fact it does so in the case of yellow fever However our concern is with reasonably normal sera Further a good general procedure to eliminate many of these agents is to determine if the inhibitory activity is associated with the gamma globulin fraction as obtained by ammonium sulfate by alcohol or most elegantly by electrophoresis If the inhibitor is not in the globulin fraction one does not have an antibody If it is in the globulin fraction one may or may not have a specific antibody which brings us to our third category of inhibitors and here matters become considerably more complex

Inhibitors that are globulins The fact that we have a neutralizing substance against the polioviruses that is heat stable and in the gamma globulin fraction does not permit us to conclude that we have an antibody stimulated by the virus of poliomyelitis There are examples of gamma globulin fractions active in the inhibition of hemagglutination test that are not even true antibodies but I am not aware of any gamma globulin fractions having neutralization activity that are not true antibodies I feel we can conclude that in the case of the neutralizing substances to poliovirus all the data are compatible with the interpretation that we are dealing with a true antibody I believe the burden of proof is upon those who would suggest an alternative interpretation If the neutralizing substance is not an antibody what is it?

However the decision that one has an antibody merely raises another question What was the antigenic stimulus? The problem that confronts us is that of serologic cross reactions for different viruses may share common antigens and give rise to different but closely related antibodies Cows have antibodies to the virus of smallpox because they had cowpox mice because they had mousepox Cows may have antibodies to the virus of psittacosis because they have been infected with the agent of sporadic bovine encephalomyelitis[6] The viruses of canine distemper and measles cross react[27] One may find antibodies to the virus of Japanese encephalitis B because an animal has been infected with the West Nile virus for we now know that there are two large families of arthropod borne viruses each of whose members are antigenically related It is quite easy to see that if we were not aware of these cross reacting viruses a variety of neutralizing substances would simply be called nonspecific inhibitors One can cite many other examples The

There are substances other than antibody that neutralize viruses There are many of them most of them poorly defined many with unusual properties These substances defy anything but a loose classification and I propose to consider them under three general headings (1) heat labile inhibitors of virus that are obviously not antibody (2) heat stable inhibitors of virus that are obviously not antibody and (3) heat stable inhibitors of viruses that may or may not be antibody

Heat labile inhibitors A variety of heat labile activities have been observed the most thoroughly studied and perhaps the most common being properdin This inhibitor has been found active against the viruses of St Louis encephalitis herpes Murray Valley fever mumps influenza and Newcastle disease [12, 13] which strongly indicates that unheated sera can be a source of considerable confusion However the nonspecific activity of unheated sera can be removed simply by heating sera to 56° C for 30 min I cannot too strongly emphasize that in any serologic survey sera should first be heated for this immediately eliminates a large number of nonspecific inhibitors There are many studies in the literature that cannot be interpreted because the authors failed to heat their sera or failed to indicate whether or not the sera were heated There is one recorded error subsequently corrected by the author[14] in which antibodies to Newcastle virus were found regularly in human sera until the sera were heated when the antibodies promptly disappeared There is some uncertainty about always heating sera for in the case of certain viruses higher neutralizing titers have been reported with unheated sera We may cite as examples neutralization tests done with the viruses of western equine encephalitis dengue and mumps [15, 18] However there is reason to believe that such unheated sera do not offer a more sensitive indicator of antibody by virtue of the additional presence of complement but rather a summation of two distinct inhibitory effects one being antibody and the other a heat labile inhibitor perhaps properdin Heating may thus lower the observed activity but it probably defines the antibody more precisely Although the heat labile inhibitors may be important in resistance to infection little more need be said about these agents from our particular point of view for heating to 56 C for 30 min immediately removes them from consideration as antibodies Certainly then our inhibitor in bovine sera is not properdin or any other heat labile substance

Heat stable inhibitors that are not globulins We use the term heat stable to refer to any inhibitory activity that remains after heating sera to 60 C for 30 min A variety of serologically reactive heat stable nonantibody inhibitors have been described in the sera from many animal species and observed under several serologic conditions in the complement fixation test in agglutination flocculation neutralization and in the inhibition of hemagglutination test In virology it is the specificity of the inhibition of hemagglutination test that is most commonly obscured In nearly all cases where the inhibition of hemagglutination test is used nonspecific inhibitors occur To cite a few examples (1) there is an inhibitor in normal sera[19] active against the viruses of influenza and it must be removed with RDL (receptor destroying enzyme) sodium periodate or trypsin [20] (2) there is a large particle in sera that inter

related to human viruses, for they are neutralized by human gamma globulin and there is clearly an area of animal and human relationships to be worked out with the bovine viruses

Discussion and summary There are a large number of unknown inhibitors in animal sera, and there is no simple method that allows us readily to identify these agents We have discussed procedures that will help more precisely to define the antibody status of these inhibitors and they are summarized below

To determine whether an inhibitor is an antibody for our test organism the following observations are helpful

(1) Sera should retain their inhibitory activity after heating at 60 C for 30 min and the inhibitory activity should be in the globulin fraction It may also be helpful to heat the sera to 80 C for 20 min, since antibodies would be inactivated at this temperature while heat stable nonantibody inhibitors such as lysozyme would remain active

(2) Inhibitors should show serologic specificity and titers should vary among animals with a pattern of passive transfer and an increase in incidence with the age of the animal

(3) If the inhibitor is a true antibody the titer should not be affected when sera are treated with RDE sodium periodate trypsin or fat solvents Centrifugation at high speed should not remove the inhibitor

(4) If sera are positive by one serologic procedure they should be tested by other serologic procedures for example if sera positive by the inhibition of hemagglutination reaction also give a positive complement fixation or neutralization reaction one has good evidence of an antibody

(5) A booster response of the inhibitor when the animal is injected with the test organism is also evidence for a true antibody

(6) If available clinical and epidemiological data with a rise in inhibitor titer are almost conclusive evidence of a true antibody

It should be noted that some of these suggested procedures are not always feasible and one may have a true antibody without being able to demonstrate all of the above reactions, for example an antibody may be demonstrable by the neutralization test but not by the complement fixation test More over one may have a nonspecific inhibitor that possesses some of the above properties for example resistance to the agents listed in the third observation cited above However I think the guide is useful for a nonspecific inhibitor would have very few of the above properties while a true antibody may possess most if not all of the above reactions

Finally though one may have an antibody that neutralizes a test virus proof that the test organism was also the original antigenic stimulus requires that one isolate the suspected virus from the animal species Only thus can the problem of cross reactions be resolved

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point to be emphasized is that cross reactions confuse our serologic data perhaps more than we realize We have only a very limited concept of the total number of cross reacting species, for a systematic study of cross reacting patterns among the thousands of bacterial and viral species of man and animals would involve an astronomical number of tests The fact that even in germ free animals antibodies can apparently develop against orally ingested dead bacilli suggests the enormous range of antigenic stimuli that daily bombard our immune mechanism [8] There is no way of knowing how many of our unknown inhibitors represent cross reacting antibodies though I feel that they should loom very large in our consideration of the meaning of unknown inhibitors

On the basis of the serologic evidence alone one then can merely say that this is an antibody that reacts with the test organism and two interpretations remain (1) the antibody is due to a stimulus received from the polioviruses or (2) from an antigenic relative of the polioviruses A precise answer can be obtained only by the isolation of the responsible agent

We have been carrying out extensive isolation studies in an attempt to discover the origin of these antibodies to the polioviruses We have not yet discovered it but I might summarize our study briefly As in the case of other investigators we have found feces a fertile source of viruses in calves overwhelmingly so We have isolated over 75 viruses from the feces of apparently normal calves and cows These agents are not yet classified We do not know how many distinct species are in the group though there are certainly several on the basis of their cytopathogenic effects in calf kidney tissue culture All but two of the viruses were isolated from young animals in the age group 6 months to 2 years and it is quite clear that in humans as in calves there is a period of intense viral infection early in life

None of these 75 viruses are agents of human poliomyelitis quite simply because they all grow in calf kidney tissue culture and we have never succeeded in getting any of the 3 strains of poliovirus to grow in this medium There remains the question as to whether any of these viruses are antigenic relatives of the agents of poliomyelitis What we have done is to screen these agents against sera containing antibodies to Types I II and III polioviruses In an as yet incomplete study we found 2 bovine agents that were neutralized by pooled heated sera However normal heated monkey sera were also found to neutralize our bovine viruses to approximately the same titer (1 16) and so obviously we did not have any bovine relatives of the polioviruses

In order to avoid the problem of unknown inhibitors in monkey sera we tried to immunize rabbits with our bovine strains and polioviruses for rabbits do not commonly have normal inhibitors to polio Coxsackie or ECHO viruses [9]

However we found that rabbits also have a heat stable inhibitor that neutralized several of our bovine viruses in titers up to 1 32 These inhibitors apparently prevented any significant antibody response in spite of rather intense antigenic stimulation We are currently working on the problem of finding a suitable animal for immunization purposes

In an incomplete survey we do know that two of our bovine viruses are

COUNTERPARTS OF HUMAN VIRAL DISEASE IN ANIMALS

By Hilary Koprowski
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Introduction

Every fool believes what his teachers tell him, and calls his credulity science or morality as confidently as his father called it divine revelation.[1]

Animal counterparts of diseases occurring in humans offer a fruitful approach to experimental studies of such entities in the animals, leading to a better understanding of the human counterparts. For obvious reasons, large-scale experimentation with humans as subjects is not feasible, although a great deal of knowledge has been obtained in the past by studies in volunteers or by heroic experiments performed by scientists on themselves. One of the most recent examples that can be cited is the study of the Coxsackie and adenovirus groups of viruses by the deliberate exposure of volunteers to the agent or agents isolated from subjects in the course of illness. Infectious hepatitis is another entity that has baffled all attempts at thorough study because of the lack of suitable animal hosts, and the knowledge gained thus far has been based almost entirely on studies on voluntary participants.

While experiments can be conducted in volunteers with diseases that run a mild course, great caution must be exercised in the case of diseases that might endanger the lives of the volunteers. For the most part, this limitation does not apply to animals. Here the field is open to pursue study of the clinical syndrome encountered in nature and to attempt to induce its counterpart by exposure of genetically homologous species to the agent or agents isolated in the field. The time factor is an additional advantage in the use of animals, as the period required for study of a new disease entity is considerably shorter.

The purpose of this paper is to present in a schematic form aspects of animal diseases for comparison with the human viral entities that can be considered as their counterparts. For apparent reasons, such comparison of necessity must be based more on the clinical symptomatology and pathology than on etiology. This approach no doubt presents many pitfalls; the knowledge gained from naturally acquired or experimentally induced diseases in animals can serve only as an adjunct to our understanding of the possibly similar entities in humans.

There is a wealth of wisdom in the terse admonition addressed by Koch to his students and which they have passed down to us, advice which is pertinent even today and should be kept in mind by all students of virology: *Meine Herren, vergessen Sie nie, dass die Mäuse keine Menschen sind.* *

Infections of the Central Nervous System

There are numerous human counterparts of animal encephalitides. This group of illnesses has probably the least to offer from the standpoint of con

* Gentlemen, never forget that mice are not human beings.

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TABLE 2

EXAMPLES OF INFECTIONS OF MAN THAT OCCUR IN ANIMALS USUALLY IN LATENT FORM AND THAT ARE INTERTRANSMISSIBLE

Virus	Vector	Species affected	Symptomatology
St Louis	Mosquito Mite	Chicken Man	None Encephalitis
Yellow fever	Mosquito	South American primates Man	Occasional liver involvement Liver involvement
LCM*	?	Mice Man	None Meningitis pneumonia

* Lymphocytic choriomeningitis

In Brazilian jungles moribund marmosets were found to be infected with yellow fever virus but whether the South American primates constitute a natural reservoir of the virus has yet to be proved The ubiquitous nature of the lymphocytic choriomeningitis virus is well known to students of disease Mice have been chosen as an example of an animal host simply because we know thanks to Traub[7] and to Armstrong Wallace and Ross[8] more about their role than those of the other hosts The symptomatology in man is variable and the sporadic nature of the infection is quite apparent The disease may occur in man in virulent form[9] but these cases are probably exceptions to the usual latent infection

Poliomyelitis like Diseases

The poliomyelitis like diseases constitute a strange group and originally they were grouped together (TABLE 3)[10] New studies of the Teschen disease seem to indicate that it does not resemble human poliomyelitis as closely as it was originally thought Although it can be induced by the ingestion of infectious material according to Horstmann[1] the virus is strangely absent from feces Because of its ubiquity in outbreaks among animals it is impossible to determine the ratio of symptomatic cases to latent forms and also to calculate accurately the mortality ratio which seem to be higher than in the case of human poliomyelitis Furthermore the disease of the animal takes on a more acute form than does the pathologically similar condition in man

TABLE 3

THE STRANGE CASE OF POLIOMYELITIS LIKE DISEASES OF ANIMALS AND MAN

V[illegible]	S[illegible] [illegible]ted	N[illegible]	T[illegible]	[illegible] feces	[illegible] CNS
Teschen	Swine	?	Feeding	—	Encephalomyelitis
Theiler	Mice	?	Feeding	++	Anterior horn
Polio	Man	Con ?	Feeding	++	Anterior horn and brain stem

TABLE 1

EXAMPLES OF INFECTIONS OF CENTRAL NERVOUS SYSTEM FOR WHICH COUNTERPARTS ARE FOUND IN HUMAN MEDICINE AND WHICH ARE INTERTRANSMISSIBLE

Virus	Natural hosts	Vector	Species affected	Symptomatology	Site of [illegible]
Arbor A	Birds	Mosquito	Horses Mules Man	Encephalitis Encephalitis Encephalitis	Brain Brain Brain
Japanese B	Birds??	Mosquito	Horses Swine Man	Encephalitis *Encephalitis* Encephalitis	Brain *Brain* Brain
Louping ill	??	Tick	Sheep Man	Encephalitis Encephalitis	Brain Brain

Arthropod borne encephalitides serologic group A data refer chiefly to the North American equine encephalitides

troversy The pathways of the infectious process are well known the etiological agents are recognized and intertransmissibility between man and animals is well defined However, the natural reservoir of infection in some cases still presents a problem

Among members of the Group A arthropod borne (Arbor) encephalitides[3] the eastern and western equine encephalitis viruses have been most thoroughly investigated (TABLE 1) Man the accidental host is infected by the same vector as are the animals The incidence course and outcome of the human illness are similar to those of horses and mules The third member of the Arbor A group Venezuelan equine encephalitis is highly contagious for man and can be transmitted either by a vector[3] or by direct contact with infected fomites[4] Strangely this virus which is highly virulent for horses and mules seems to provoke a rather mild type of infection in humans who acquire it probably by inhaling the infected material[5] The only known fatal case occurred in a man who became infected *in nature* during an outbreak of the disease among horses[3] It may be questionable to speculate on the slight statistical evidence of one case as to whether the course of human illness is conditioned by the manner in which this infection is acquired Relatively little is known about the other members of the Arbor A group

The pathway of infection with the Japanese B virus course of illness and outcome are similar in man and animals The relationship between louping ill and Russian spring summer encephalitis viruses is very close and human beings are known to have become infected with these agents These viruses are transmissible between different species and cause disease of similar symptomatology

The diseases thus far discussed are known to be the cause of frequent epizootics of quite severe character but on TABLE 2 are shown examples of infections that usually remain latent in their animal hosts When transmitted to man they may cause either severe illness or inapparent infection The tremendous variation in the clinical picture of human St Louis encephalitis may be cited as an example[6]

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Teschen	Swine	?	Feeding		Encephalomyelitis
Theiler	Mice		Feeding	++	Anterior horn
Polio	Man	Cow	Feeding	++	Anterior horn and brain stem

Theiler's virus infection of mice runs a course similar to that of human poliomyelitis. Infectivity of fecal material and lesions in the anterior horn of the spinal cord are factors that suggested the classification of this disease as mouse poliomyelitis. In this connection it is worthwhile to mention that the mouse-adapted human polioviruses do not behave at all similarly to the true mouse poliomyelitis virus. The latter causes intestinal infection and is excreted during a prolonged period, whereas the mouse-adapted strains can infect their murine hosts only when introduced directly into the central nervous system.

There is no immunological relationship between the agents causing poliomyelitis-like disease of swine, mice, and man; whether some animal acts as a reservoir for human polioviruses has not yet been determined. Bartell and Klein[12] and Morton Klein (elsewhere in these pages) have called attention to the possible role of poliovirus antibodies in the sera of cattle. In this connection I should like to relate a curious experience which, if confirmed, may throw more light on the problem. Five calves 4 weeks old were placed under observation in separate stalls. Once a week, stools were collected from each animal and a blood sample was obtained. Suspensions of the fecal material were inoculated into tissue cultures of monkey renal epithelium; from one sample obtained after 4 weeks of observation there was isolated a virus which was cytopathogenic for tissue cells (FIGURE 1). This virus was subsequently identified as Type I human poliovirus. Results of the neutralization test performed against Type I standard poliovirus serum of the same animal indicated the presence of Type I antibodies at the time of virus isolation. The concentration of antibodies was low, but the difference in titers seemed to be insignificant. In order to exclude the possibility of laboratory contamination during the process of isolating the virus, the same experiment was repeated using the same stool suspension as source of virus. Again Type I poliovirus was isolated. It is quite obvious that many more factors must be elucidated before absolute confirmation of this curious incident may be obtained. I have cited this experience here in order to encourage other investigators to search

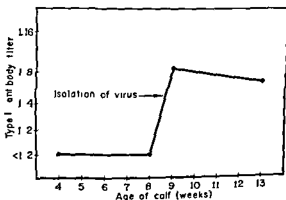


FIGURE 1. The isolation of poliovirus Type I from the feces of a calf.

for evidence which if confirmed may well point to cattle as hosts for another human pathogen in moderate form

Hepatitis of Man and of Animals

Today many infectious diseases are suppressed both epidemiologically and financially However infectious hepatitis of man stands out as an important disease entity the etiological agent of which thus far has defied the efforts of the most zealous investigators Although it has been isolated by passage in human volunteers failure to find for it a suitable animal host and to develop specific serologic reactions seriously impedes progress in the study of this disease

There are several hepatotropic agents that cause hepatitis syndrome in animals and 4 of these will be discussed briefly (TABLE 4) Three viruses causing hepatitis of mice were detected almost simultaneously in 3 laboratories in 3 different strains of mice[13 15] One strain was isolated from I RI mice suffering from leukemia[15] There was a tremendous variation in the susceptibility of mice of different strains to induced infection with this agent The murine hepatitis virus (MHV) of Gledhill and Andrewes[14] can produce fatal hepatitis in mice only in association with *Eperythro oon coccoides*[16] The analogy with the role of *Hemophilus influenzae* in swine influenza infection is quite obvious An association of a normally harmless parasite with a moderate virus resulting in a virulent form of hepatitis has not been observed in other hosts

Although Rift Valley fever infection of man runs a dengue like course its virus is hepatotropic for sheep and cattle Focal necrosis of the liver follows a fulminant type of infection in young lambs In man the morphologic changes following infectious hepatitis are variable but postnecrotic cirrhosis seems to be the most prevalent

TABLE 4
EXAMPLES OF INFECTIOUS HEPATITIS OF ANIMALS AND OF MAN

Spe	T m	Lat y—day	I b i —day	F tal ty rat	A t fi l t t th m pe	S l g d t ty w th
Mouse	Contact	Probably life long	5 to 8	*	None	None
Sheep and cattle	Mosquito	?	1 to 3	>20 10	Mice hamsters monkeys etc	Rift Valley fever infectious for man†
Dog	Contact	161	6 to 9	10	Foxes tissue *culture*	Fox encephalitis
Duck	Contact	Unknown	1 to 3	35 to 85	None	None
Man	Contact	>480	20 to 40	0 5	None	None

* None if murine hepatitis virus (MHV) alone i injected approaching 100 per cent if together with *Eperythro oon coccoides*
† Apparently not hepatotr pic in man denguelike symptomato o y

Comparisons between canine and human infectious hepatitis have been reported by qualified observers and need not be repeated here. If only the etiological agents of these rather similar syndromes were intertransmissible, methods of prevention seemingly successful in canine hepatitis could then be applied to the human disease.

Hepatitis of the duck is the newest member to be added to the hepatotropic family.[17-19] Although different in character from the human disease, one common feature was observed: the protective effect of antibody-containing serum which appears to be similar to that of gamma globulin administered to man.[20]

Respiratory Viruses

The sense of frustration hounding the author while playing the "counterparts" of human viral diseases attacking the respiratory tract, was in no small measure occasioned by the prevailing ignorance of the characteristic features of many clinical entities, compounded by our lack of knowledge of the numerous agents isolated in the course of respiratory illnesses of either man or animal which are still "in search of a disease." The naming (or rather misnaming) of newly discovered entities only adds to the confusion.

For example, there is nothing characteristic about pneumonia of man caused by filtrable agents. The roentgenographic findings vary widely in character and distribution, and the pulmonary lesions reveal an interstitial type of bronchopneumonia sometimes limited to one lobe, sometimes involving two. This condition may be encountered in psittacosis, Q fever, smallpox, chicken pox, measles, and lymphocytic choriomeningitis. In addition, there are 4 other diseases, including the so-called atypical pneumonia, in which this ubiquitous bronchopneumonia is seen—diseases caused by an agent or agents unknown. Finding a counterpart of this human disease in animals is like equating an unknown with an unknown. There are certainly many conditions which clinically and pathologically resemble human viral pneumonias; one of these, distemper, may cause pneumonia in dogs. The problem of cattle pneumonia is even more complicated, since it appears that almost every disease of cattle, particularly of young calves, is characterized by diarrhea and ends in pneumonia (TABLE 5). Calf pneumonia refers to a syndrome first described by Baker[1] in 1942. It is characterized by bilateral pneumonia of the anterior and

TABLE 5

INFECTIONS OF THE RESPIRATORY TRACT OF ANIMALS WITH THEIR HUMAN COUNTERPARTS (STRETCHING THE POINT OF COMPARISON)

Virus	Animal type	Counterpart in human disease
Distemper	Dog	Atypical viral or Q fever pneumonia
Calf pneumonia	Cattle	Atypical viral or Q fever pneumonia
Pink eye	Horses	Adenovirus infections
Bullnose	Swine	Common cold
Rhinotracheitis	Cattle	If mild, resembles common cold
Snotsiekte	Cattle	If very mild, resembles common cold

ventral lobes and occurs mostly during the winter months It is apparently caused by an agent that can be adapted to the mouse lung

In their severe forms infectious rhinotracheitis of Colorado[23] and the upper respiratory disease of California[22] will also cause bronchopneumonia These two conditions apparently caused by immunologically identical agents in their mild form may resemble a very severe type of common cold However can a disease with a fever of 104 1 accompanied by excessive salivation accelerated and shallow breathing and anorexia still be classified as a common cold?

There is no dissolution of the turbinate bones in the human common cold and therefore rhinitis of swine is not a counterpart for this condition Bullnose is believed to be a mild form of the disease characterized by swelling of the mucosa without subsequent distortion of the snout Whether this resembles the condition of the snout in the common cold is a question that must be left to the imagination rather than to science

It was difficult to find even a single counterpart for the numerous conditions caused by the adenoviruses in man The illness of horses referred to in TABLE 5 is really equine influenza However if we call it pink-eye and stress that the conjunctivitis is accompanied by a catarrhal syndrome we may achieve some success in comparing the two entities However it should be mentioned that in pink-eye occasionally the function of one or both eyes may be lost and sometimes pneumonia causes death This is not the case in adenovirus infections

Gastrointestinal Infections

In a complicated field such as that comprising the so-called virus infections of the gastrointestinal tract our ignorance is truly encyclopedic Although the alternate problems of diarrhea and constipation are probably as important to man as to animals my feeling is that we are wallowing in a morass of unknowns *The diarrhea of suckling mice*[24] *is a bane to every laboratory engaged in mouse* breeding (TABLE 6) The explosive outbreaks of this illness often ruin carefully planned experiments in which young mice are to be used as experimental hosts

TABLE 6
INFECTIONS OF ANIMALS PRIMARILY AFFECTING THE GASTROINTESTINAL TRACT

Species	Age at onset	Fatality rate per cent	Factors affecting	Characteristics	Etiology
Mouse	10 to 15 days	0 to 100	Age season strain	Inclusion bodies	Virus?
Cattle	1 to 2 days	Nearly 100	Age season colostrum feeding	—	CPE virus
	Up to 9 months	*0*	*Age season colostrum feeding*	*Elementary bodies*	Psittacosis group
	All ages	4 to 8	Not defined	—	Virus ?
Swine	<10	100	Age	—	TGE† virus
	All ages	0		—	

Calf pneumonia enteritis
† Transmissible gastroenteritis

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Respiratory Viruses

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For example there is nothing characteristic about pneumonia of man caused by filtrable agents The roentgenographic findings vary widely in character and distribution and the pulmonary lesions reveal an interstitial type of bronchopneumonia sometimes limited to one lobe sometimes involving two This condition may be encountered in psittacosis Q fever smallpox chicken pox measles and lymphocytic choriomeningitis In addition there are 4 other diseases including the so called atypical pneumonia in which this ubiquitous bronchopneumonia is seen—diseases caused by an agent or agents unknown Finding a counterpart of this human disease in animals is like equating an unknown with an unknown There are certainly many conditions which clinically and pathologically resemble human viral pneumonias one of these distemper may cause pneumonia in dogs The problem of cattle pneumonia is even more complicated since it appears that almost every disease of cattle particularly of young calves is characterized by diarrhea and ends in pneumonia (TABLE 5) Calf pneumonia refers to a syndrome first described by Baker[21] in 1942 It is characterized by bilateral pneumonia of the anterior and

TABLE 5

INFECTIONS OF THE RESPIRATORY TRACT OF ANIMALS WITH THEIR HUMAN COUNTERPARTS (STRETCHING THE POINT OF COMPARISON)

V[illegible]u[illegible]	A[illegible]mal[illegible]p[illegible]	Cou[illegible]t[illegible]rpa[illegible]t [illegible]h[illegible]ma[illegible] m[illegible]d
Distemper	Dog	Atypical viral or Q fever pneumonia
Calf pneumonia	Cattle	Atypical viral or Q fever pneumonia
Pink-eye	Horses	Adenovirus infections
Bullnose	Swine	Common cold
Rhinotracheitis	Cattle	If mild resembles common cold
Snotsiekte	Cattle	If very mild resembles common cold

ventral lobes and occurs mostly during the winter months It is apparently caused by an agent that can be adapted to the mouse lung

In their severe forms infectious rhinotracheitis of Colorado[72] and the upper respiratory disease of California[73] will also cause bronchopneumonia These two conditions apparently caused by immunologically identical agents in their mild form may resemble a very severe type of common cold However can a disease with a fever of 104 F accompanied by excessive salivation accelerated and shallow breathing and anorexia still be classified as a common cold?

There is no dissolution of the turbinate bones in the human common cold and therefore rhinitis of swine is not a counterpart for this condition Bullnose is believed to be a mild form of the disease characterized by swelling of the mucosa without subsequent distortion of the snout Whether this resembles the condition of the snout in the common cold is a question that must be left to the imagination rather than to science

It was difficult to find even a single counterpart for the numerous conditions caused by the adenoviruses in man The illness of horses referred to in TABLE 5 is really equine influenza However if we call it pink-eye and stress that the conjunctivitis is accompanied by a catarrhal syndrome we may achieve some success in comparing the two entities However it should be mentioned that in pink-eye occasionally the function of one or both eyes may be lost and sometimes pneumonia causes death This is not the case in adenovirus infections

Gastrointestinal Infections

In a complicated field such as that comprising the so called virus infections of the gastrointestinal tract our ignorance is truly encyclopedic Although the alternate problems of diarrhea and constipation are probably as important to man as to animals my feeling is that we are wallowing in a morass of unknowns The diarrhea of suckling mice [4] is a bane to every laboratory engaged in mouse breeding (TABLE 6) The explosive outbreaks of this illness often ruin carefully planned experiments in which young mice are to be used as experimental hosts

TABLE 6
INFECTIONS OF ANIMALS PRIMARILY AFFECTING THE GASTROINTESTINAL TRACT

Sp	Ag at set	F tal ty rat p c t	F t d ff t g	Cha t l t les	Et l gy
Mouse	10 to 15 days	0 to 100	Age season strain	Inclusion bodies	Virus?
Cattle	1 to 2 days	Nearly 100	Age season colostrum feeding	—	CPE virus
	Up to 9 months	0	Age season colostrum feeding	Elementary bodies	Psittacosis group
	All ages	4 to 8	Not defined	—	Virus ?
Swine	<10	100	Age	—	TGE† virus
	All ages	0		—	

Calf pneumonia enteritis
† Transmissibl gastroenteritis

Comparisons between canine and human infectious hepatitis have been reported by qualified observers and need not be repeated here If only the etiological agents of these rather similar syndromes were intertransmissible methods of prevention seemingly successful in canine hepatitis could then be applied to the human disease

Hepatitis of the duck is the newest member to be added to the hepatotropic family [17-19] Although different in character from the human disease, one common feature was observed the protective effect of antibody containing serum which appears to be similar to that of gamma globulin administered to man [20]

Respiratory Viruses

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Table

Attempts to Represent in the Least Confusing Way the Current Status of Viral Diarrheal Disease of Man

Age	Syndrome	Transmission experiments with stool filtrates		
		Species	Symptom	Serial passages
Newborn	EDN*	Calves ?	Diarrhea ?	29
Children	SDI†	Rabbit	Corneal inflammation	Several
All ages	ENG‡	Man	Diarrhea	8
		Cat	Diarrhea	Several

* Epidemic diarrhea of newborn
† Stomatitis and diarrhea of infants
‡ Epidemic nonbacterial gastroenteritis

by nausea, anorexia, vomiting, abdominal spasms and watery stools.[37] Filtrates of stools fed to human volunteers caused a similar condition in the hands of several groups of workers[37,38] and a claim was made that the agent was transmitted serially when administered orally to a cat.[39]

It is obvious that the comparative pathology of gastrointestinal viral infections is still in its teens and that many years will pass before we may benefit from studies of these conditions in animals in order to improve our knowledge of their human counterparts.

Infections Inducing Fetal Damage

Infections that result in fetal damage constitute perhaps the most fruitful field of research in comparative pathology. Stringent biological and ethical considerations that limit research studies in man are more than amply compensated by the occurrence of similar syndromes in animals. These may occur in the course of a naturally acquired disease, or they may be induced by agents biologically active against the fetus *in utero* but not after parturition.

Enzootic abortion of ewes is such an example of a latent viral infection. The dormant agent resembles psittacosis virus[40] (TABLE 8). It would seem that pregnancy may help to make the infection manifest, resulting in abortion late in gestation. The apparently normal lambs born of infected ewes harbor the latent virus for eight months or more.[41] Development of a vaccine protecting the ewes against abortion has been claimed.[42]

Some of the ewes injected during the first thirty days of gestation with the so-called attenuated bluetongue virus will give birth to lambs that show arrested development of the central nervous system and the attendant characteristic malformations.[43] Apparently this agent is effective only on a fetal host, as no infection can be induced after parturition. A similar condition was observed by Young[44] in sows when they were injected with an attenuated leporine strain of hog cholera virus during the first month of gestation. The German measles

Strangely the disease does not affect newborns but only mice 10 to 15 days old In some outbreaks all mice may die within 48 hours in some cases the animals recover but their growth is retarded, and there are also instances of complete recovery Pathological lesions are limited to the ileum and colon and intranuclear inclusion bodies were found in the epithelial cells of the villi which resembled those observed in infectious panleukopenia of cats[24] The peak of the disease occurs in winter and its incidence declines in warm weather a situation quite unlike that presented by the human counterpart of a similar ailment The genetic constitution of the host plays an important role in susceptibility to infection and it is conceivable that genetically controlled breeding could lead to the establishment of a resistant strain of mice

In contrast to mouse diarrhea the agent of calf pneumonia enteritis (CPE) has been isolated[6] and transmitted to susceptible newborn animals in an aerosol form which is apparently the only satisfsactory way to transmit the disease[7, 8] The sharp change in the resistance to the infection with increase in age makes the condition somewhat similar to the epidemic diarrhea of newborn babies (EDN) which apparently can be transmitted to calves The omnipresent psittacosis virus seems to be one of the agents causing diarrhea of cattle[9] the agent was always present in feces of experimentally infected animals and could be isolated from different parts of the intestinal tract As in the case of its predecessor (TABLE 6) colostrum feeding had a protective effect

The next diarrheal entity of cattle is referred to as all ages disease In 1956 it consisted of three immunologically distinct subentities two named for reasons of local patriotism as virus diarrhea of New York and virus diarrhea of Indiana while a third was topographically called simply mucosa disease[30] Although diarrhea is the characteristic feature of the disease oral erosions and lamination complete the syndrome The fatality rate of 4 to 8 per cent is quoted for the New York entity The mortality rate of the Indiana diarrhea is apparently much higher in the mucosa disease it appears that all of the affected animals die[30] Although I have listed this condition I am not familiar with a human counterpart in which man would become lame because he had ten stools per day

The transmissible gastroenteritis (TGE) of swine is as well defined as it possibly can be in this confused field The explosive start of the disease in baby pigs characterized by vomiting profuse diarrhea with yellowish stools resembling curdled milk[31, 32] followed by extreme dehydration resembles greatly the neonatal syndrome in babies first described by Rice[33]

Unfortunately whereas the etiological agent of TGE has been isolated immunologically defined[32] grown in tissue culture and possibly attenuated[34] the identity of the EDN agent of man still remains uncertain (TABLE 7) Although passaged serially in calves and immunologically identified[35] it was apparently not tested for its disease producing faculty in newborns and it may be considered as perhaps only one component of a complex etiological entity Even less is known about stomatitis and diarrhea in infants (SDI)[36] The syndrome has been described in various outbreaks but the identity of the agent passaged on rabbit cornea has not been firmly established

Epidemic nonbacterial gastroenteritis (ENGE) is a syndrome characterized

TABLE 9

VESICULAR DISEASES OF ANIMALS AND MAN

Virus	Incubation days	Mucosal lesion	Site affected	No. of virus types	Convalescing immunity (months)
Foot and mouth	2 to 14	Vesicle without areola	Lips Mouth	7	6 to 24
Vesicular stomatitis	2 to 14	Vesicle without areola	Tongue Pharynx	2	6 to 24
Vesicular exanthema	2 to 14	Vesicle without areola	Stomach Coronary band of hoofs	7	6 to 24
Herpangina (Coxsackie A)	2 to 9	Papules or vesicles with red areola	Soft palate Tonsils Pharynx	7	0 (Permanent)

the formation of scars The site of lesions in the oral cavity is similar for all four of these conditions but of course lesions of the coronary band of hoofs remain characteristic for the animal pathogen It is interesting to note the multiplicity of the virus types that cause the same illness Seven types of foot and mouth virus are matched by the same number of vesicular exanthema agents[45] and Coxsackie A strains causing herpangina[49] Degenerative heart muscle changes observed in foot and mouth disease have not been described for Coxsackie A viruses but they were observed in newborn infants after Coxsackie B infection[50, 51] Another difference lies in the observation of the length of immunity produced the fleeting type of convalescing immunity is typical for animals whereas a more lasting type of immunity follows Coxsackie A infection

Papillomas

No self respecting writer on problems in pathology would dare to close without mentioning some aspect of tumor investigations There are animal viruses causing cancers or leukemia that cannot be considered as counterparts of nonexisting forms in humans However papilloma will save the day (TABLE 10)

The local epithelial hyperplasia which occurs in crops in different locations of the human body[52] is due to a virus that causes formation of cytoplasmic and intranuclear inclusions and may be seen apparently under an electron microscope[53] Attempts to infect other species with the human virus have failed Conversely the filtrable agent of bovine papillomatosis was supposedly transmitted to man by Schultz in 1908[54] I was unable to find any confirmation of this observation but the bovine material was reported to cause tumor formation in horses[55]

Equine and canine papillomatoses greatly resemble their bovine counterpart and in both instances species specificity of the causative agent was maintained The Shope papilloma of the rabbit has been so well described that there is no need for repetition However one may mention that this virus when passed

TABLE 8

EXAMPLES OF INFECTIONS OF ANIMALS CAUSING DAMAGE TO THE FETUS AND THEIR COUNTERPARTS IN HUMAN MEDICINE

Virus	Species	Results of infections during pregnancy	Time of attack
Ovine enzootic abortion	Sheep	Abortion or normal birth	Late in gestation
Attenuated bluetongue	Sheep	Malformation (crazy lambs*)	Early in gestation
Equine rhinopneumonitis	Horse	Abortion stillbirth or normal	Late in gestation
Leporine hog cholera	Swine	Abortion stillbirth malformation	Early in gestation
German measles	Man	Abortion stillbirth malformation†	Early in pregnancy

* Arrested development of central nervous system
† Blindness deafness cardiovascular abnormalities

syndrome in man presents a similar picture with abortions, stillbirths and malformations

The identities of the viruses of equine abortion and of equine influenza are still uncertain[45, 46] but their effect on the fetus is similar to that induced by the agent of ovine enzootic abortion occasional stillbirths but also frequent normal births, and/or abortion late in gestation

Concerning these conditions one might ask this provocative question Are the animals born from virus infected females immunologically normal? Will exposure to the same agent encounter increased resistance or increased susceptibility because of the inability to develop antibodies? In other words does the mechanism of immunological tolerance operate in these fetal virus infections? In the case of ovine enzootic abortion it apparently does not

German measles is of ill omen for a woman during the first trimester of pregnancy[47] American statistics indicate that abortions stillbirths and malformations consequent to this disease are probably as frequent as normal births The direct toxic role of the agent has not yet been proved and studies in animals would doubtless help to clarify the entire problem

I may add that obviously there are other viral infections of pregnant animals which may cause abortions stillbirths and malformations However the four examples cited above are so characteristic and bear so much more upon the problem of the human syndrome that the others were omitted from consideration

Vesicular Diseases

It was not easy to find counterparts in human medicine to the three vesicular diseases of animals As is well known the host range and type of lesions do not permit any easy differential clinical diagnosis of the three agents foot and mouth disease vesicular stomatitis and vesicular exanthema (TABLE 9) The mucosal lesions are similar to those observed in herpangina of man minute vesicles filled with clear fluid which in the case of foot and mouth virus rupture and leave denuded areas In the case of vesicular stomatitis vesicular exanthema and herpangina the vesicles heal usually promptly and without

TABLE 11

EXAMPLES OF LACK OF COUNTERPARTS

Animal disease with no known human counterpart	Human disease with no known animal counterpart
Pseudorabies Scrapie	Exanthematous diseases, roseola infectious mononucleosis, etc

It is an intriguing thought that perhaps one day children will be vaccinated with distemper virus against measles, dogs with rinderpest virus against distemper, and cattle with measles virus against rinderpest. *Se non è vero è ben trovato.* *

No Counterparts

As a *gran finale* to this disquisition the following table is presented to show viral diseases for which I was unable to find any counterparts (TABLE 11). The two animal diseases are characterized by an itch. The only human affliction conceivable as a counterpart is the seven year itch, and that is a much more pleasant condition, although it is perhaps exasperating at times, than the animal syndrome.

Conversely, animal skin seems to be devoid of ingredients that would make it susceptible to the exanthema with which the human species is blessed during every viral disease of childhood. Thus, if chicken pox is the price we must pay for enjoying the seven year itch, we should accept this bargain with all humility, for the poor sheep, while undergoing a three year incubation period of scrapie,[63] are not recompensed with a delightful itch.

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* If it is not true, it is well invented. (GIORDANO BRUNO)

Table 10
Papillomas of Animals and Man

Species	Location	Demonstration of virus
Cow	Head neck	Filtrates induce papilloma in cattle man(?) and horse(?)
Horse	Nose lips	Transmission to horses
Dog	Mouth lips	Filtrates induce papillomas in dogs
Rabbit (cottontail)	Neck shoulders abdomen	Shope papilloma
Man	Palms and soles	Transmission by filtrates to man electron microscopy

into domestic rabbits but not to cottontails gives rise to papillomas that subsequently may undergo malignant transformation [56] This has not been observed to my knowledge in case of human warts but these can be treated by psychotherapy a method not as yet applicable to cottontails

New Relations Measles Distemper and Rinderpest

For the past several years suspicions had been harbored that the distemper virus may possibly be pathogenic for man Appearance of distemper antibodies in sera of children recovering from unidentified infections of the upper respiratory tract inclined scientists[57, 58] to believe that an immunological kinship of the two conditions might exist [59] in recent years the presence of distemper antibodies in the blood of man has been confirmed by Karzon[60] and by Carlstrom [59] The presence of antibodies in relation to age of the donors revealed a pattern commonly encountered in other viral infections of man newborn infants showed high incidence of antibodies which dropped to insignificant levels by the age of 6 months but from the second year incidence rose rapidly paralleling the age curve and antibodies were present in all of the sera of adults studied [59, 61]

The relationship between the presence of distemper antibodies and measles infection of man was established by Carlstrom [61] who found at least a fourfold rise in distemper antibody titer in sera of 11 of 14 patients convalescing from measles In addition there was an evident correlation between the history of measles and the appearance of distemper antibodies in 37 out of 38 patients It is quite obvious that these observations require further investigation to determine the true nature of this most interesting relationship

To complicate the picture further a preliminary report from workers in Kenya Africa would seem to indicate a possible protective effect of induced asymptomatic rinderpest infection against distemper challenge [62] Four test puppies pretreated with rinderpest virus showed no signs of distemper when challenged with distemper virus whereas all four control puppies developed signs of distemper and one of them died Here too confirmation of this relationship should be sought

COMPARATIVE STUDIES ON VIRAL HEPATITIS IN ANIMALS AND IN MAN*

By Morris Pollard

University of Texas Medical Branch, Galveston, Texas

Studies with human viral hepatitis have been handicapped by the lack of a susceptible experimental animal and of a specific diagnostic test. This was amply documented at a recent symposium on the laboratory propagation and detection of the agent of hepatitis (National Academy of Sciences–National Research Council, Washington, D. C., 1954). A subsequent report on the propagation of human viral hepatitis agents in the Detroit 6 strain of human epithelium (Rightsel *et al.*, 1956) has not yet been confirmed. The significance of the cytopathogenic effect noted in the latter publication must yet be supported by human infectivity trials with the agents being propagated.

The embryonated chicken egg has been used as a medium for the propagation of hepatitis agents; however, thus far adaptation of hepatitis viruses to the chick embryo has not been proved (Henle, 1954). While hepatitis agents have induced no demonstrable lesions in the embryonated egg, recent observations indicate that influenza and mumps viruses induce an increased number of mononuclear cells in the chorioallantoic fluid (CAF) of inoculated but asymptomatic embryos (Pollard and Diserens, 1955; Buchner *et al.*, 1956). Serum specimens from acute cases of human viral hepatitis appeared to induce a similar response (Pollard and Diserens, 1956); however, this has not yet been confirmed (Buchner *et al.*, 1956).

Since the virus or viruses of hepatitis in man are not transmissible to experimental animals (MacCallum, 1954; Morris, O'Conner and Coburn, 1954), since they induce questionable changes in cultures of human tissues, and since they bring about controversial changes in the embryonated egg and indistinct serologic reactions, it was deemed advisable to study the characteristics of a laboratory prototype of hepatitis virus. Some useful information from this might be applicable to the laboratory study of viral hepatitis of man.

Murine viral hepatitis shows some resemblance to the human disease (Friend, Wroblewski and LaDue, 1955; DeRitis, Coltorti and Giusti, 1956). It shows a narrow species susceptibility. It has not been propagated in fertile eggs (Gledhill, Dick and Niven, 1953) and its effect in tissue cultures is uncertain. We are indebted to A. W. Gledhill, National Institute for Medical Research, Mill Hill, London, England, for kindly providing us with a strain of mouse hepatitis virus (MHV) that was free of *Eperythrozoon coccoides* (Niven, Gledhill and Andrewes, 1952). This virus was lethal for newborn mice, usually killing them at 6 to 8 days following intra-abdominal inoculation, and causing extensive degenerative lesions in the liver. We are grateful to J. B. Nelson, The Rockefeller Institute for Medical Research, New York, N. Y., for

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Since the virus or viruses of hepatitis in man are not transmissible to experimental animals (MacCallum, 1954; Morris, O'Conner and Coburn, 1954), since they induce questionable changes in cultures of human tissues, and since they bring about controversial changes in the embryonated egg and indistinct serologic reactions, it was deemed advisable to study the characteristics of a laboratory prototype of hepatitis virus. Some useful information from this might be applicable to the laboratory study of viral hepatitis of man.

Murine viral hepatitis shows some resemblance to the human disease (Friend, Wroblewski and LaDue, 1955; DeRitis, Coltorti and Giusti, 1956). It shows a narrow species susceptibility. It has not been propagated in fertile eggs (Gledhill, Dick and Niven, 1953) and its effect in tissue cultures is uncertain. We are indebted to A. W. Gledhill, National Institute for Medical Research, Mill Hill, London, England, for kindly providing us with a strain of mouse hepatitis virus (MHV) that was free of *Eperythrozoon coccoides* (Niven, Gledhill and Andrewes, 1952). This virus was lethal for newborn mice, usually killing them at 6 to 8 days following intra-abdominal inoculation, and causing extensive degenerative lesions in the liver. We are grateful to J. B. Nelson, The Rockefeller Institute for Medical Research, New York, N. Y., for

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TABLE 2
COMPARATIVE TITRATION OF MHV IN CHICK EMBRYOS AND IN NEWBORN MICE

	Virus dilution				
	10^{-1}	10^{-3}	10^{-}	10^{-4}	10^{-}
MHV in chick embryos	40	15	4	12	2
MHV in chick embryos	29	7	8		
Above 61 C /40 minutes	6				
MHV in newborn mice	9/9†			6/7	
Above 61 C /40 minutes	0/11				

* Histiocytes per 100 oil immersion fields Normal level 0 to 15
† Number of mice infected/number inoculated

sheets of cells developed within 5 days The nutrient fluid was poured off and 0 1 ml of MHV (Princeton PRI) virus was added to each tube of tissue culture Ten minutes later 0 9 ml of maintenance solution (calf serum 3 per cent in Eagle s BSS) was added to each tube The maintenance solution (MS) was changed at 3 or 4-day intervals however, the cultures were observed microscopically every day Newborn mice were inoculated with base line culture fluid and with homogenized tissue in the MS at 5-day intervals Cytopathology was not detected during the 15 days of observation and MHV was not recovered from any specimen except for the base line inoculum (TABLE 3) This experiment was repeated with L-strain cells but cultures were tested at earlier intervals (TABLE 4) MHV was recovered from the culture 3 hours after inoculation and at 9 hours 1 of 6 inoculated mice developed the disease

TABLE 3
MHV (NELSON PRI) 1 5 DILUTION IN L STRAIN CELL CULTURE

Time after inoculation	Cytopathology	Mouse inoculation
Base line	—	5/5
Five days	0	0/5
Ten days	0	0/5
Fifteen days	0	0/5

Number of mice infected/number of mice inoculated

TABLE 4
MHV (NELSON PRI) 10^{-} IN L-STRAIN CELL CULTURE

Time after inoculation	Cytopathology	Mouse inoculation
Base line	—	6/6
3 Hours	0	5/5
9 Hours	0	1/6
25 Hours	0	0/4
25 Hours—no tissue	—	0/4
48 Hours	0	0/5

Number of mice showing liver lesions/number of mice inoculated

kindly providing us with a strain of MHV (PRI strain) which was also used in this study The PRI virus (Nelson, 1952) produces a more fulminant infection in weanling mice in which extensive liver degeneration is manifested

Three aspects of mouse hepatitis virus are reported on here (1) the effect of MHV in the embryonated chicken egg, (2) the fate of MHV in cultures of mouse tissues, and (3) the extraction of a complement fixing antigen from MHV infected mouse livers

(1) Pooled livers from mice acutely infected with MHV (Gledhill strain) were homogenized in a Ten Broeck grinder with Hank s balanced salt solution (BSS) (10 per cent by weight) The emulsion was centrifuged at 2000 rpm for 30 minutes, then at 10 000 rpm for 20 minutes The supernatant fluid of the latter was then placed in ampoules and stored at dry ice temperature for future use The MHV, thus prepared was inoculated into the chorioallantoic fluid (CAF) of 7 day old embryonated eggs After 4 days of further incubation the inoculated eggs were chill killed by overnight storage at 4 C and the cell content of pooled CAF from these eggs was determined microscopically The technique has been described elsewhere (Pollard and Diserens 1955) Control inoculations consisted of MHV heated at 56 C for 30 minutes, 'normal liver emulsions prepared according to the sequence for MHV above, and CAF from uninoculated embryonated eggs Each egg inoculated with MHV received approximately 2000 newborn mouse LD_{50} MHV was titrated simultaneously for infectivity in newborn mice and for cytological response in the CAF of embryonated eggs

Infective inocula of MHV were associated with increased cells in CAF of inoculated embryos (TABLE 1) Cell counts following inoculations with heat inactivated virus and normal liver extracts were within normal limits, as were the counts from the uninoculated eggs When the MHV inoculum was diluted beyond 10^{-1} there was no significant cellular response in the CAF however virus effect was still evident in mice at 10^{-4} dilution (TABLE 2) Heat inactivated MHV was again noninfectious for newborn mice and negative in chick embryos Passage of egg material from the 10^{-1} dilution inoculum into additional eggs was negative

(2) The L strain of mouse fibroblast (Earle *et al* 1943) was propagated in stationary tubes with 20 per cent horse serum in Hank s BSS Continuous

TABLE 1

COMPARATIVE EFFECT OF MOUSE HEPATITIS VIRUS (MHV) BY HISTIOCYTE RESPONSE IN EGGS AND BY INOCULATION INTO NEWBORN MICE

Inoculum	Histiocytes per 100 field	Mouse cul ti*
MHV—liver emulsion	85	25/25
MHV 56 C /30 minutes	22	0/8
MHV 12 liver emulsion	120	5/
MHV 56 C /30 minutes	12	0/2
Normal liver emulsion	15	0
Uninoculated control	15	0

Mice infected/mice inoculated

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TABLE 3

MHV (NELSON PRI) 1:5 DILUTION IN L-STRAIN CELL CULTURE

Time after inoculation	Cytopathology	Mouse inoculation*
Base line	—	5/5
Five days	0	0/5
Ten days	0	0/5
Fifteen days	0	0/5

* Number of mice infected/number of mice inoculated.

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MHV (NELSON PRI) 10^{-} IN L-STRAIN CELL CULTURE

Time after inoculation	Cytopathology	Mouse inoculation*
Base line	—	6/6
3 Hours	0	5/5
9 Hours	0	1/6
25 Hours	0	0/4
25 Hours—no tissue	—	0/4
48 Hours	0	0/5

* Number of mice showing liver lesions/number of mice inoculated.

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Three aspects of mouse hepatitis virus are reported on here (1) the effect *of MHV in the embryonated chicken egg (2) the fate of MHV in cultures of* mouse tissues, and (3) the extraction of a complement fixing antigen from MHV infected mouse livers

(1) Pooled livers from mice acutely infected with MHV (Gledhill strain) were homogenized in a Ten Broeck grinder with Hank s balanced salt solution (BSS) (10 per cent by weight) The emulsion was centrifuged at 2000 rpm for 30 minutes then at 10 000 rpm for 20 minutes The supernatant fluid of the latter was then placed in ampoules and stored at dry ice temperature for future use The MHV thus prepared, was inoculated into the chorioallantoic fluid (CAF) of 7 day old embryonated eggs After 4 days of further incubation the inoculated eggs were chill killed by overnight storage at 4° C , and the cell content of pooled CAF from these eggs was determined microscopically The technique has been described elsewhere (Pollard and Diserens 1955) Control inoculations consisted of MHV heated at 56 C for 30 minutes 'normal liver emulsions prepared according to the sequence for MHV above and CAF from uninoculated embryonated eggs Each egg inoculated with MHV received approximately 2000 newborn mouse LD_{50} MHV was titrated simultaneously for infectivity in newborn mice and for cytological response in the CAF of embryonated eggs

Infective inocula of MHV were associated with increased cells in CAF of inoculated embryos (TABLE 1) Cell counts following inoculations with heat inactivated virus and normal liver extracts were within normal limits as were the counts from the uninoculated eggs When the MHV inoculum was diluted beyond 10^{-1} there was no significant cellular response in the CAF however, virus effect was still evident in mice at 10^{-4} dilution (TABLE 2) Heat inactivated MHV was again noninfectious for newborn mice and negative in chick embryos Passage of egg material from the 10^{-1} dilution inoculum into additional eggs was negative

(2) The L strain of mouse fibroblast (Earle *et al* 1943) was propagated in stationary tubes with 20 per cent horse serum in Hank s BSS Continuous

TABLE 1
COMPARATIVE EFFECT OF MOUSE HEPATITIS VIRUS (MHV) BY HISTIOCYTE RESPONSE IN EGGS AND BY INOCULATION INTO NEWBORN MICE

I ocul m	H t yt pe 100 fi ld	M l t
MHV—liver emulsion	85	25/25
MHV 56 C /30 minutes	22	0/8
MHV 12 liver emulsion	120	5/7
MHV 56 C /30 minutes	12	0/2
Normal liver emulsion	15	0
Uninoculated controls	15	0

* Mice infected/mice inoculated

TABLE 2
COMPARATIVE TITRATION OF MHV IN CHICK EMBRYOS AND IN NEWBORN MICE

	Virus dilution				
	$10^{-[illegible]}$	$10^{-[illegible]}$	$10^{-[illegible]}$	$10^{-[illegible]}$	$10^{-[illegible]}$
MHV in chick embryos	40	15	4	12	2
MHV in chick embryos	29	7	8		
Above 61 C /40 minutes	6				
MHV in new born mice	9/9†			6/7	
Above 61 C /40 minutes	0/11				

Histiocytes per 100 oil immersion fields Normal level 0 to 15
† Number of mice infected/number inoculated

sheets of cells developed within 5 days The nutrient fluid was poured off and 0 1 ml of MHV (Princeton PRI) virus was added to each tube of tissue culture Ten minutes later 0 9 ml of maintenance solution (calf serum 3 per cent in Eagle s BSS) was added to each tube The maintenance solution (MS) was changed at 3 or 4-day intervals however the cultures were observed microscopically every day Newborn mice were inoculated with base line culture fluid and with homogenized tissue in the MS at 5-day intervals Cytopathology was not detected during the 15 days of observation and MHV was not recovered from any specimen except for the base line inoculum (TABLE 3) This experiment was repeated with L-strain cells but cultures were tested at earlier intervals (TABLE 4) MHV was recovered from the culture 3 hours after inoculation and at 9 hours 1 of 6 inoculated mice developed the disease

TABLE 3
MHV (NELSON PRI) 1 5 DILUTION IN L-STRAIN CELL CULTURE

Time after inoculation	Cytopathology	Mouse inoculations
Base line	—	5/5
Five days	0	0/5
Ten days	0	0/5
Fifteen days	0	0/5

Number of mice infected/number of mice inoculated

TABLE 4
MHV (NELSON PRI) 10^{-1} IN L-STRAIN CELL CULTURE

Time after inoculation	Cytopathology	Mouse inoculation
Base line	—	6/6
3 Hours	0	5/5
9 Hours	0	1/6
25 Hours	0	0/4
25 Hours—no tissue	—	0/4
48 Hours	0	0/5

Number of mice showing liver lesions/number of mice inoculated

TABLE 5

MHV (GLEDHILL) 10^{-2} IN L STRAIN CELL CULTURE

Time after inoculation	Cytopathology	Mouse inoculation
Base line	—	10/10
25 Hours	0	0/5

Number of mice showing liver lesions/number of mice inoculated

TABLE 6

MHV (NELSON PRI) IN MINCED TISSUES FROM NEWBORN MICE
(Virus titration in base line $10^{-4.5}$)

Time after inoculation	Mouse inoculation	
	Liver tissue	Kidney tissue
9 Hours	0/6	3/3
25 Hours	0/3	3/3
72 Hours	0/3	1/3
144 Hours	—	0/2
25 Hours—no cells	1/10	

Number of mice showing liver lesions/number of mice inoculated

Tissue free virus cultures were negative when tested after 25 hours at 37° C. A repeated experiment with MHV (Gledhill strain) was negative at 25 hours and cytopathology was not observed (TABLE 5).

MHV (Princeton PRI) was inoculated into flask cultures of minced liver and minced kidney tissue in MS. The initial virus content of the MS showed mouse infectivity out to LD_{50}—$10^{-4.5}$. MHV was not recovered from the liver tissue cultures when tested at 9, 25, 72 and 144 hours. MHV was recovered from the kidney cultures at 9 and at 25 hours after inoculation and 1 of 3 mice developed hemorrhagic liver lesions from a 72 hour culture inoculum. The tissue free virus control killed 1 of 10 mice inoculated after 25 hours at 37° C (TABLE 6). It might be argued here that the liver tissue may not have survived for 9 hours thus affording little medium for virus survival; however liver tissue imprints at this time showed histologically intact cells. If MHV entered the mouse cells and manifested an "eclipse" phase in virus reproduction, then either this phase is longer than 15 days or it was extinguished.

During the past year 24 acute serum specimens from viral hepatitis patients have been studied in monolayer cultures of HeLa cell and in accord with the findings of others (Bang and Warwick, 1954; Henle, 1954; Syverton, 1954) cytopathology was not detected. The tissues were homogenized in the MS and inoculated into the CAM of embryonated eggs. All such inoculations were negative. The Detroit 6 strain of cell (Stulberg, Berman and Rudille, 1955) was kindly transferred to our laboratory by J. W. McLean of Parke Davis & Company, Detroit, Mich. It was propagated carefully according to the details stressed by McLean and his colleagues (personal communication). R. H. Bussell of our laboratory worked with this cell strain for over nine months

While it is difficult to propagate this strain was adapted satisfactorily to propagation in horse serum Serum specimens from six selected acute cases of human viral hepatitis have thus far failed to induce cytopathology in the cultures For obvious reasons we were unable to test the inoculated cultures for virus content nor were the inocula tested for virus content The inocula did induce significant cytological changes in the CAM of inoculated eggs

(3) Pools of liver tissue from newborn Swiss mice acutely ill with MHV (Gledhill) infection were homogenized 10 per cent by weight in Hank s BSS and this material was centrifuged at 2000 rpm for 30 minutes then at 10 000 rpm for 20 minutes at 4° C * Aliquots of supernatant fluid from the latter preparation were then centrifuged at 120 000 g* for 1, 2 and 3 hours The supernatant fluid was then separated and the sediment was resuspended to original volume in Hank s BSS Portions of the ultracentrifuged preparations were subjected to (1) testing for infectivity by inoculation into newborn mice (0 05 ml intraperitoneally) (2) desiccation and reconstitution in distilled water (3) desiccation and extraction with chemically pure benzene 3 times at 20 minute intervals and then to reconstitution in distilled water to its original volume (4) mixture with one third volume of diethyl ether at 4° C overnight and rendered ether free by centrifugation and vacuum and (5) heating at 61 C for 20 minutes Control antigens were processed from liver pools from uninoculated mice

Antiserum was prepared by inoculating clarified MHV (Gledhill) into adult Swiss mice (0 1 ml intraperitoneally) twice at a 2 week interval and the mice were exsanguinated 2 weeks following the last inoculation When diluted to 10^{-5} the immunizing inocula killed newborn mice

Control sera were processed from mice of the same colony just prior to the study of MHV infection and from mice inoculated with liver extracts processed from uninoculated mice

The complement fixation (CF) technique employed was essentially that described by Bengtson (1944) in which 0 2 ml quantities each of antigen and serum and 2 units of complement were used The hemolytic system was added after incubation overnight at 4° C Complement fixation was determined by visual inspection Mouse serum was diluted initially 1 10 in buffered physiological saline and heated at 61 C for 20 minutes

A box titration of MHV (Gledhill) with serum from immunized and from normal mice is outlined in TABLE 7 Extracts from normal mouse liver did not fix complement in the lowest dilutions of serum and antigen Further tests with antigens and sera were made with 16 units of antigen and with 8 units of antiserum Complement fixation tests made with the antigen preparations described above were positive with the supernatant fluid of the preparations centrifuged at 10 000 rpm and at 40 000 rpm (120 000 g) at 1 2 and 3 hours (TABLE 8) A quantitative decline in CF antigen in the supernatant fluid of the latter procedure was detected following heating ether extraction desiccation and desiccation with ether extraction however these procedures did not cause complete extinction of antigenicity The sediment

* A model L preparative Spinco centrifuge was used

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Part III Leptospirosis A Contemporary Problem

INTRODUCTION

By Donald S Martin
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It was just fifty years ago that Stimson[1] described the finding of organisms now accepted as lepto pires in the kidneys of a patient who died supposedly of yellow fever in New Orleans La A few years later the organi m was cultivated successfully from cases of Weil s disease by investigators working independently in Japan[2] and in Germany [3]

Then as in many diseases a period of discovery was followed by a lull *Leptospira icterohaemorrhagiae* was recognized as the etiological agent of Weil s disease the epidemiology was worked out and the textbooks confidently printed the facts that the organism was carried by rats and that man acquired his infection when he became contaminated by rat urine

During the past fifteen to twenty years the entire picture has changed leptospirosis is now recognized as a world wide public health problem of increasing importance We now know that the term leptospirosis does not connote a single di ease entity and that the clinical pictures may be quite different depending upon the particular serotype causing the infection We know also that leptospirosis of domestic animals is of great concern to farmers milk producers and breeders of cattle and swine

The various clinical and diagnostic problems of the leptospiral infections were discussed at length in a symposium held five years ago at Walter Reed Medical Center Washington D C The information has been accumulating so rapidly that it is considered most appropriate that we be brought up to date once more

I take this opportunity to compare the development of our knowledge of leptospirosis with that of the systemic mycoses Until the last few decades coccidioidomycosis and histoplasmosis were considered as rare but almost always fatal infections with quite characteristic clinical features Today it is known that infections from these fungi are extremely common in certain areas of the country and that the development of a full blown fatal form of either disease is the exception Infections from these fungi result in such a variety of clinical manifestations that the true epidemiology of the disease became apparent only when diagnostic skin and serologic tests were developed and applied in large scale studies It was necessary to impress bacteriologists with the fact that they should not throw out the cultures after the first two or three days—it was necessary to alert physicians to suspect a fungus infection in a patient who did not respond to antibiotic therapy

I feel that the same principles are applicable today in leptospirosis Both bacteriologists and physicians must be alerted always to consider the possibility of leptospirosis in any patient with an obscure febrile illness Blood cultures must be taken early in special media and must be held for weeks

Leptospirosis cannot be excluded because the patient shows no jaundice or denies falling in water contaminated by rat urine New leptospiral serotypes pathogenic to man are being found in other parts of the world and the clinical picture of infections caused by these serotypes may be quite different from that of classic Weil s disease canicola fever pomona meningitis or pretibial fever We now are learning also that leptospires of various serotypes can be cultivated from an ever widening range of animal species

The possibilities are just beginning to unfold and the reader will find in the present section of this monograph the latest information on leptospirosis which is becoming of increasing significance to those who are concerned with infectious diseases of both man and animals

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ANIMAL RESERVOIRS OF LEPTOSPIRES

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Man forms a purely incidental and nonessential link in the chain of the epidemiology of leptospirosis As a rule the human infection is exhausted within itself and it is only in extremely rare instances that it is the cause of fresh cases of the illness

Furthermore the pathogenic *Leptospira* are not able to adapt themselves to a saprophytic life and are unable to reproduce in the outside world in which their survival is somewhat limited in time

Consequently the exclusive sources of the infection direct or indirect are the animal carriers and shedders of the *Leptospira* A knowledge of these is essential for the determination of the epidemiological characteristics of the infection and in order to be able to provide in advance really efficient prophylactic measures

It is necessary to define clearly what is meant by the term carrier In the first place one must distinguish between the condition of the occasional and temporary shedder of leptospires and that of the true carrier The first is a normal condition for every individual who suffers or has recently recovered from an infection by leptospires During the illness there is constant leptospiruria which continues long after the recovery but invariably, only for a limited period generally for several weeks

The temporary shedder of leptospires may constitute a source of natural infection but his epidemiological importance is relatively limited not only because the leptospiruria ceases after a short time but also because in handling the sick or convalescent patient it is easy to carry out the appropriate rules of prophylaxis

The condition of the permanent carriers is quite different Here one is dealing with animals that have suffered infection by leptospires (an infection that may have shown morbid manifestations or that may have remained symptomatologically blank) and have then remained shedders of leptospires via the urine for a long period or even for the rest of their lives It is these carriers that permit the survival of individual serotypes of *Leptospira* and that constitute the most dangerous source of the infection

Our knowledge of the leptospire carriers is not yet complete The carriers among the more common animals which more easily come into contact with man are sufficiently well known Knowledge is much less plentiful however with regard to the rarer animals that are more difficult to study experimentally and are less close to man In fact at some times when one finds leptospires in the urine or kidney of such animals one does not know whether one is dealing with temporary shedders or with true carriers

From a practical point of view the importance of the animal carriers depends on different factors One of these is the reaction of the urine It is well known that the pathogenic leptospires are very sensitive to variations of *p*H

Research that I have carried out on some serotypes of these has shown that the extreme limits of pH that may permit the survival of leptospires for a period of at least 6 days in an environment otherwise favorable to their existence are between 6 24 and 8 23 In order that the survival be prolonged to one month these limits must be reduced to 6 35 and 7 96 Some animals including man have a distinctly acid urine and in their case the leptospires excreted with it are either dead or at least unable to reproduce the illness In such cases and for this reason the animal in spite of being a carrier, does not constitute a danger from contagion The reaction of the urine may vary, however even in the same animal, according to its diet

This is the case in the dog which if given a diet of meat has an acid urine fatal for the leptospires when however the dog has a predominantly vegetable diet it has a urine of neutral or even slightly alkaline reaction much more favorable to the survival of the leptospires According to Zuelzer[1] the rat also generally has a urine with a pH of 5 4 to 5 8 but if it subsists primarily on vegetables the pH rises to a level of 7 to 8

Generally the herbivorous animals have a neutral or slightly alkaline urine while that of the carnivores is usually acid It is also necessary to remember that an acid diet at least in dogs does not succeed in disinfecting the animal The leptospires that colonize the small renal canals survive and reappear in virulent form in the urine as soon as its reaction has again become favorable

It must also be remembered that leptospires although very sensitive to an acid environment are nevertheless able to survive in it at least within certain limits for a very short period Thus an animal that excretes a moderately acid urine may be dangerous if the urine is immediately mixed with water or mud of favorable reaction In this case the leptospires surviving the brief transit in the acid urine find themselves in environmental conditions favorable to their existence

Another factor of great importance is that of the environment in which the animal carrier lives Rarely does the infection occur directly through contact with the infected urine Man nearly always infects himself indirectly by way of water mud or foods contaminated by the urine Consequently animal carriers that have the capacity for contaminating their surroundings are particularly important those that eliminate leptospires but disperse them in an unfavorable environment where they rapidly die out are of little consequence For example the importance of small rodents that live in the flooded rice fields is very different from that of the jackal which in Israel lives in arid or semi desert regions

Another example is given by the conditions which in the plains of the large rivers of central and eastern Europe permit in some years the outbreak of epidemics of the so-called *Schlammfieber* (mud fever) caused by *L grippotyphosa* The usual carrier of this leptospire is the field mouse (*Microtus arvalis*) that lives in holes scattered throughout the fields

If the season is dry the illness caused by *L grippotyphosa* is slight because the leptospires eliminated with the urine have little chance of surviving in such surroundings When however the season is rainy and especially when after great river floods the plains remain muddy and dotted with puddles for

a long time the earth becomes saturated so to speak with leptospires This condition gives rise to the epidemics of *Schlammfieber* which sometimes affect untold thousands of persons who work in such fields

Finally a third factor of great practical importance is the possibility of contact direct or indirect between the animal carriers and man It is easy to comprehend how wild animals that live far from human habitations and cultivated land have much less epidemiological importance if they are carriers than those that live in homes in stables or in the cultivated fields and especially those that experience frequent manual attention

These three factors variously combined among themselves condition the practical importance of the different animal carriers of leptospires

The Relation Between Leptospires and the Organism of the Animal Carrier

What is the mechanism through which the quality of the carriers is established? It is not yet completely known and it still presents difficulties similar to those encountered when the same problems are examined in the field of the bacterial carriers

In the first place it is worth remarking that at least as far as is known at present the condition of the carrier is not established unless the animal has suffered an infection by leptospires This infection may give evident clinical manifestations or may pursue its course completely undetected In both cases however during the course of the infection a transitory state of leptospiremia exists In this stage it is possible to find leptospires in all organs including the kidney Leptospires are found in the interstitial tissue in this organ as well as in the vessels However it is only in relatively small numbers and often simultaneously with more or less extensive lesions of nephritic nature that they penetrate into the renal canals and pass into the urine When on the other hand an immunized state is established in the patient which happens after seven to ten days the leptospires disappear from the blood and the interstitial tissue and begin to accumulate in the secondary convoluted tubules These masses true colonies of leptospires adhere to the epithelial cells and sometimes are even included in them without exerting any evident pathogenic action on them with the exception in some cases of a mechanical action that produces a flattening of the epithelia themselves[2] The masses are sometimes very large being constituted of an inextricable tangle of spirochetes It does not seem however that they ever succeed in completely obstructing the tubules in which they colonize a fine canal in the center always persists through which the urine may pass

Numerous leptospires detach themselves from such masses and are then transported by the urine to the exterior It may be said that at this stage the leptospires are transformed from internal parasites of the organism into external parasites and that in a sense their life approaches that of the saprophytes At this point the leptospires are released from the action for them harmful of the defense mechanism of the host The antibodies circulating in the blood and in the tissues do not succeed in reaching them and are not even able to exert an antigenic action on the host In fact in animal carriers

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the antibody level gradually diminishes at a rate equal to that in noncarriers until it reaches a level no longer detectable by our methods of investigation During a search for *L ballum* in numerous specimens of *Apodemus sylvaticus* that I carried out in Spain four strains of these leptospires were isolated all from mice that did not show any trace of antibodies in the serum On the other hand antibodies were found to be present in other mice that however were not carriers The same phenomenon has been observed in cattle by Gillespie *et al*[3] and by Kmety *et al*[6] in dogs by Alexander *et al*[4] in pigs by Burnstein and Baker[5] and in rodents by numerous other authors[7-10]

However as already mentioned some points are still obscure One of these arises from the fact that the leptospires are able to survive in the renal tubules even though antibodies against them are present in the urine[11,12] However it is true that such antibodies have a low titer and never reach the high level that may be found in the serum

On the other hand it is known that if a specific serum of not very high titer is introduced into a culture of leptospires one nearly always observes a certain number that are able to survive and multiply giving place by the phenomenon of agglutination to masses that in a sense may even call to mind those observed in the renal tubules

Another obscure point is the reason for the establishment of the state of the permanent carrier in certain cases while in others the urinary elimination of the leptospira is only a transitory event Some obscure phenomena of biological affinity certainly exist here by these a state of biological equilibrium is easily established between some serotypes of leptospires and some animal species This equilibrium is either lacking or much more difficult to establish in other cases Both components of this symbiotic association play a part in this phenomenon In fact it is observed that while certain animals easily become carriers of some serotypes of *Leptospira*, they do not become so for others This confirms anew the biological validity discussed by some investigators of the subdivision of *Leptospira* into serotypes, at the same time it demonstrates the fact that the distinction made initially between temporary shedders and persistent carriers although unconventional is one that rests on a solid if poorly explicable biological basis Any animal susceptible to infection by leptospires may become a temporary shedder but only those animal species that present a particular condition of biological sympathy for a determined serotype of *Leptospira* can become carriers For example the rat which is a habitual carrier of *L icterohaemorrhagiae* is unable to become one for the serologically related *L canicola*[14]

It should be pointed out that even within the ambit of the same species not all the animals that have suffered infection necessarily become carriers this occurs only in some Why this occurs it is difficult to explain and it is also difficult to understand why some animals remain carriers all their lives while others after a period of months or even years become autodisinfected However these phenomena are not only peculiar to the leptospire carriers but are common to all the other categories of carriers of pathogenic agents

Furthermore even the different strains of *Leptospira* belonging to the same serotype behave differently in the same host Van der Hoeden[15] has pointed

out that the white mouse may become a carrier of *L grippotyphosa* on condition that it is inoculated with a sufficient quantity of this agent The animal may then eliminate the leptospires with the urine either continuously or intermittently according to the strain of the agent

Animals that are carriers of leptospires do not seem to suffer any damage from their condition[16-18] It is true that partial renal lesions have been described in some carriers (occasionally in cattle and more often in dogs) but it is unlikely that these lesions are to be attributed to the leptospires that the carrier harbors it is more probable that they are the consequence of the previous acute infection by leptospires In fact these same lesions may also be found in cattle and in dogs that have recovered from leptospirosis but have not become carriers

On the other hand one has the impression that the circumstance of being harbored by one particular animal species rather than by another does exert a certain influence on the pathogenic property of some leptospires In this respect clear experimental results are lacking nevertheless it is probably true that as noted by some authors the transit of *L icterohaemorrhagiae* in the rat accentuates its virulence Thus *L bataviae* disseminated in Italy by the harvest mouse *Micromys minutus soricinus* almost invariably produces a light anicteric infection whereas in Indonesia where the principal carrier is the rat *Rattus diardi* it causes a serious illness in which jaundice is very often present

In the same series of phenomena may be included the fact—one I have repeatedly observed —that when man contracts an infection of *L pomona* by direct contact with pigs he almost always becomes ill with distinct and prevalent meningitic symptoms whereas these are very often absent in cases of infection due to the same leptospire if it is contracted in the country where it may be acquired from a different source

Although it is generally accepted that the animal carrier of leptospires harbors these spirochetes in the kidney and eliminates them with the urine the hypothesis has nevertheless been advanced that the leptospires may persist in some other organs for example in the brain or the salivary glands This latter hypothesis has been advanced because of the repeated observation of infections by a leptospire following a bite from a dog or more frequently from a small rodent[19 21] This hypothesis is probably untenable Thus far it has never been demonstrated with certainty that a chronic animal carrier of leptospires may present them in the saliva or the salivary glands[21] The cases recorded above may be explained either by supposing that the biting animal happens to be in the acute stage of the infection in which case the leptospire may indeed be found in the saliva or by assuming that the wound caused by the bite has been contaminated by infective urine excreted by the animal This contingency is probable in cases in which the bite has been received from a mouse caught with the hands since it is well known that these small rodents easily excrete urine when frightened

Another question is that of when and why an animal becomes a carrier or to be more precise contracts the infection

It is noted from the research of Uhlenhuth and Fromme[22] that leptospires

are able to pass through the placenta and to reach the fetus. Sixteen years ago Biocca and I[23] were able to show that sometimes this produced neither abortion nor death of the newborn, but simply an illness of short duration in the parent. *Nevertheless it has never been demonstrated that an animal* has become a carrier through an infection contracted during the fetal stage. On the contrary, the observations of all workers occupied with this topic agree that in rats and mice the percentage of carriers is much higher in adult animals than in the young. This indicates that, at least in these animals, the infection by leptospires usually occurs neither during the fetal period nor during the period of infancy, when closer contact with the parents, possible carriers, would suggest that contagion might be easier. It may be that newborn animals acquire from the mother, through the placenta or with the milk, a temporary passive resistance that protects them for a time. In addition, it must not be forgotten that in very young animals leptospirosis takes a more severe course, so that many of these victims die before becoming carriers.

In any case all the evidence seems to suggest that the animals more often become infected when they are adults and in the same way in which man may become infected. Furthermore, it is probable that the infection may occur *through copulation. It has been experimentally proved,* in fact, that leptospires may pass through the genital mucosa; moreover, some cases of human infection by sexual contact have also been described.[4, 5]

Leptospira in the Arthropoda

The possibility that insects might be carriers of the agent of Weil's disease was considered even before the genus *Leptospira* was discovered and its etiological role recognized. Some epidemiological data made the following hypothesis appear probable. The frequency of the illness in persons living in marshy zones, its greater diffusion in the summer, and the influence of rain and humidity on the oscillation of the illness are all factors that were assumed to cause the epidemiology of Weil's disease to approach that of malaria. This theory was supported particularly by Hecker and Otto[26] and the insects mainly suspected were the mosquito and the horsefly.

Later, after the discovery of *Leptospira,* this theory was abandoned, even *though numerous research workers attempted to demonstrate its worth* experimentally. These researches have been particularly well summarized and discussed by Uhlenhuth and Fromme,[7] who made a notable contribution to them.

The possible *role of Hematophagus flies has been studied by Keiter and* Ramme,[2] who show that *Haematopota pluvialis* may transmit leptospires to guinea pigs mechanically. The same possibility has been demonstrated by Uhlenhuth and Kuhn[2] for *Stomoxys calcitrans.* This horsefly may transmit the illness for a period as long as six days after having bitten a sick animal. In flies, however, multiplication of the ingested leptospire is not demonstrable and, according to workers who have carried out these experiments on guinea pigs, the results are not immediately relevant to man nor to the natural epidemiological conditions.

Numerous researches have been carried out on the various species of mos

quito generally with negative results[30-36] The only exception is Noguchi[37] who maintains that *Aedes aegypti* may be a carrier and transmitter of leptospires According to the unconfirmed observations of Cay and Sellards[38] leptospires are able to survive for at least 22 days in the body of the mosquito

Attempts to transmit leptospires by means of lice have likewise been unsuccessful[31 39] Positive results however have been reported by Dietrich[40] both with the louse and with a leech and by Blanchard Lefrou and Laigret[41] with bedbugs These arthropods have succeeded in infecting the guinea pig even after a period as long as 38 days subsequent to being fed on a sick human Bonne[42] however has not been able to confirm these last results

The experiments carried out on fleas and ticks by Huebner and Reiter produced negative results[39] Recently however it has been demonstrated conclusively that ticks may become infected with leptospires and may not only conserve them for a long time (at least 518 days) in their internal organs[43 44] but may also emit them with their coxal liquid and pass them on to their descendants[45]

Of particular interest are the observations of Burgdorfer[44] who not only succeeded in transmitting leptospirosis to guinea pigs by feeding infected examples of *O turicata* on them but has also found a tick infected in nature by *I ballum* These findings were recently confirmed by two Russian authors Krepkogorskaia and Rementsova[46] who were able to isolate two strains of *L grippotyphosa* from specimens of *Dermacentor marginatus* captured in the Kazakh Soviet Socialist Republic a region where leptospirosis is endemic in cattle

These researches should be worth following up because although it is very improbable that ticks may have importance in the human epidemiology of leptospirosis it is not impossible that they may play a role in the transmission of the infection from animal to animal

With the exception of these last results it may be said that all these researches were carried out for the most part in the first years that followed the discovery of the *Leptospira* when the epidemiology of the infection was still imperfectly known and that they have not produced any positive results of practical interest Certainly it must be admitted that in particular experimental conditions some arthropods may become infected with leptospires may maintain them for a reasonably long time in their bodies and may finally emit them in such condition as to render the infection of a mammal possible This is however a rare occurrence it can happen only under very particular circumstances or in well-organized experiments but it very improbably occurs in nature

Vertebrate Carriers of Leptospires

Very little research has been directed to determine the receptivity of amphibians to leptospires Chetti[47] and Corrales[48] have obtained negative results in the frog and I have searched unsuccessfully for pathogenic leptospires in some species of amphibian caught in the rice fields of Italy Uhlenhuth and Fromme[7] have found leptospires in the intestine of the frog but these are probably water leptospires that are not pathogenic

Nothing is known of the possible receptivity of fish to infections from leptospires Altava Barrera and Villalonga[49] have searched without success for pathogenic leptospires in the organs of minnows (*Gambusia*) living in the infected waters of rice fields in Spain Dohmen[50] reports the case of a fisherman who became ill with leptospirosis after being bitten by a pike but probably it was not the fish that infected him directly the wound caused by the bite formed a point of entry for leptospires present in the water

As far as I am aware research on reptiles in this connection is nonexistent

The possibility that birds may be susceptible to infection with leptospires and thus become carriers has already been examined by Uhlenhuth and Fromme[7] who have established that chickens and sparrows are resistant to the infection Martin and Pettit[51] Ghetti[47] and Oba[52] have also had negative results with chickens and pigeons and Oba[5] has proved that there are no formations of antibodies in the inoculated chicken Corrales[48] on the contrary has shown that the serum of the normal chicken exerts a lytic action on leptospires

In contrast to these results other more recent workers have succeeded in finding in nature chickens that show a high titer of agglutinins in the serum (Gillespie *et al*[3] for *L pomona* Terskich[53] for *L icterohaemoglobinuriae bovis* and *L bovis palestinensis* Kujumgiev[54] for *L andaman A* Mochmann[55] for *L icterohaemorrhagiae L grippotyphosa* and *L canicola* and Bernkopf and Olitzki[56] for *L icterohaemorrhagiae* and for *L bovis* chickens ducks geese, and turkeys) Seropositive geese have been reported by Mochmann[55] (*L grippotyphosa*) and by Vissotski and Redkina[57]

The fact that the chicken embryo may be experimentally infected with leptospires has already been demonstrated by Hallauer and Kuhn[58] and it has been shown that the chick may also be infected and may maintain leptospires in the circulation for several days (Hoag Gochenour and Yager[59]) As a result chicks are sometimes used for the isolation of leptospires from polluted material

Gillespie *et al*[3] claim to have shown experimentally that the receptivity of the chicken to infection by leptospires is limited only to the first few days of life However Bernkopf[60] has succeeded in infecting chickens older than six weeks with *L bovis* (*L grippotyphosa*) producing in them either a morbid state or an obvious tendency toward immunity The same result has been obtained in chickens and ducks by Kenzy and Gillespie[61] with *L pomonae* Chalquest[6] has succeeded in infecting experimentally with *L pomonae* not only chickens but also ducks pheasants and partridges However he has not succeeded in finding leptospires in their intestines

It has not been possible thus far to isolate a pathogenic leptospire from a bird infected in nature with the exception of the recent discovery of Kujumgiev[54] who claims to have isolated a strain of *L andaman* from the kidney of a goose This discovery however requires confirmation

The first reliable discovery of this type was made this summer by my collaborator E Bussinello in the rice fields of the Po valley in Italy Bussinello (unpublished data) has succeeded in isolating two strains of *L bataviae* from the intestine and liver of two wading birds frequently found in these rice fields Other *Leptospira* strains not yet classified have been isolated from the kidney

liver and intestine of the following species of aquatic birds *Ardella minuta* *Ardea cinerea* *Herodias ga ella* *Hidrochelidon nigra* and *Gallinula chloropus*

It is to be noted that *L bataviae* is the type of leptospire most frequently found in the rice fields of Italy where it is usually transmitted by a small mouse *Micromys minutus soricinus* It may be considered that the birds became infected from contaminated water of the rice fields or from devouring mice that were carriers of *Leptospira* Regarding the discovery of leptospires in the intestine it should be remembered that in birds the ureter flows into the intestine (cloaca)

It is not yet possible to determine whether these bird are true carriers of this leptospire or are only temporary shedders

If it should be demonstrated that they are carriers the discovery would have very great importance not only because it would modify our knowledge of the epidemiology of leptospirosis but also becau e it might explain the strange phenomenon that the same serotype of *Leptospira* may be found in widely separated localities in spite of having as habitual carriers animals that live in relatively limited areas For example *L ba aviae* is found as frequently in Italy as in some Scandinavian countries and in Indonesia It is also to be noted that the Grallae which live in the Italian rice fields are migratory birds that travel very great distances

Although it is possible that leptospires do infect other animals it is never theless beyond doubt that they are essentially parasites of mammals It is among the mammals in particular the rodents that we find their most important carriers

Undoubtedly the gray rat *Epimys decumanus* is the most important carrier Uhlenhuth and Fromme[77] have already indicated this rodent as the international carrier of *L icterohaemorrhagiae* Wherever researches are carried out it is found with rare exceptions that a notable percentage of rats sometimes 90 per cent in adult animals are carriers and shedders of *L icterohaemorrhagiae* When it is considered that the gray rat is extremely common that it is found throughout the world that with ships it can easily migrate from one country to another and that it has close contacts with man and his foodstuffs it is easy to comprehend how *L icterohaemorrhagiae* is so universally diffused and is of prime importance among the other leptospires both for the frequency and the gravity of the disease it provokes

The frequency of infection by *L icterohaemorrhagiae* among sewer cleaners among people bathing in sweet water close to sewer outlets among workers in the rice fields of Spain and in all places where rats abound shows the close relationship that exists between the presence of this animal and human infection

Also interesting in this respect are the observations made in rice fields in Spain by Altava Barrera and Marin[63] who have noted that apart from wide variation in the number of rats that infest rice fields considerable annual variations also exist in the percentage of rats that are leptospire carriers These percentages may already be determined in the spring before work begins in the rice fields and they give a very reasonable indication of what will be the development of leptospirosis among workers in the following summer If

the percentage of rats that are carriers is low there will be only sporadic cases of illness, if on the other hand it is high an epidemic is to be expected

Another example of the relation existing between the abundance of rats and the frequency of human infection is given by the epidemic of leptospirosis which, in 1951, hit Kodiak and several other localities in Alaska a territory that until then had been almost free from leptospirosis The epidemic was studied by W H Gaub (unpublished data) and also described in a United States Navy report [5]

It happened that during the course of World War II the United States armed forces had accumulated in several localities in Alaska very abundant convoys of provisions thus favoring multiplication of rodents At the end of the war these deposits were demobilized and abandoned In consequence the rats found there undisturbed an abundant source of food that permitted them to multiply at an extraordinary rate When the provisions suddenly became exhausted, the rats poured en masse into the small towns of the region and principally through contamination of foodstuffs originated a very considerable number of cases of leptospirosis

Finally it is worth noting that according to a publication by McKinley,[64] in the Philippine Republic (in which at least until 1928 human cases of leptospirosis had not been reported) the percentage of rats that are carriers is less than 1 per cent, even in cities such as Manila which have a large exchange of traffic with Japan where the percentage of carrier rats is about 50 per cent and where human cases of the infection are numerous

The rat represents the classic example of an animal carrier of leptospirosis It becomes infected at early maturity (the percentage of young rats that are carriers or even simply seropositive is very low) and as has also been shown experimentally it remains a shedder for years practically throughout its life [65, 66]

The rat may become a carrier of other pathogenic leptospires as well as *L icterohaemorrhagiae* for example *L pomona* [67, 68, 69, 71] *L ballum* and *L bataviae* [7, 73] These are only occasional discoveries however of minor practical interest

The black rat *Epimys rattus* is likewise a carrier of *L icterohaemorrhagiae* but less frequently For example researches carried out in Japan[74] have shown that almost 40 per cent of the specimens of *Epimys decumanus* are carriers of *L icterohaemorrhagiae* while scarcely 2 per cent are carriers among *Epimys rattus* This lesser frequency together with the fact that *Epimys rattus* is notably more rare than *Epimys decumanus* serves to place it in lower order of practical importance among the carriers of *L icterohaemorrhagiae*

Other rats having importance as carriers of leptospires include *Epimys diardi* the principal disseminator of *L bataviae* in Indonesia *Epimys brevicaudatus*, carrier of *L autumnalis* and of *L pyrogenes* and *Epimys culmorum* carrier in Australia of *L australis A*

Mice are also important carriers of leptospires However according to some authors [75] mice unlike rats are able to eliminate leptospires for a very long time but normally do not do so all their lives At a certain period and by a still unknown mechanism they become autosterilized

Among the mice that are carriers of leptospires the most important is *Microtus arvalis* habitual carrier of *L grippotyphosa* the leptospires most widely diffused in the great river valleys of Central and Eastern Europe The number of these mice is subject to notable annual oscillations When a year of great abundance of mice coincides with abundant rainfall and still more with floods there is nearly always a human epidemic It is necessary for these two conditions to coincide in order to make possible an epidemic Thus in 1943 Kathe[76] observed in Silesia that the field mice were very numerous but the season was very dry Consequently, not only was the epidemic diffusion of the illness in man absent but also the possibility of infection from mouse to mouse was limited In fact scarcely 1 per cent of the mice were carriers In the year 1942 on the other hand the season was rainy and although the number of field mice was not excessive it was nevertheless noticed that 18 per cent were carriers and the human illness became an epidemic

It is known however that Kathe[4] has his own particular conception of the epidemiology of *Schlammfieber* Kathe is a supporter of the *bodentheorie* according to which pathogenic leptospires should be capable not only of surviving in the external surroundings but also when these present favorable conditions of multiplying in them According to this theory the high percentage of carrier mice would not be the origin but the consequence of a wide diffusion of pathogenic leptospires in the soil

The observations of Popp[77] made during a small epidemic caused by *L grippotyphosa* among pea pickers in Germany are also interesting In the locality where the epidemic developed Popp found that from 47 to 83 per cent of the adult field mice were carriers of leptospires In the autumn after the epidemic had subsided he no longer found carrier mice in the area and even the percentage of seropositive mice was rather low (20 per cent) Popp holds that this may be explained by presuming that a good number of the carrier mice died from leptospirosis This contrasts however with what is known of the relations that usually exist between leptospires and the animal carrier

It must also be remembered that Van der Hoeden[15] has demonstrated that the white mouse may be made a carrier of *L grippotyphosa* experimentally and that it sheds the leptospires without showing any apparent ill effects for at least eighteen months

It is true however that urinary elimination is intermittent especially for some strains of *L grippotyphosa* which may explain at least in part the results of Popp

Another species of *Microtus* the *montebelli* is the principal carrier of *L hebdomadis* the agent of nanukayami or seven day fever and of *L autumnalis* the agent of hasami or autumnal fever both very common in the Japanese countryside

Finally in Israel *Microtus guentheri* has been considered the carrier of a particular strain of leptospire called *L geffeni*[7] In the years 1949 and 1950 an enormous increase in the numbers of this mouse in the country regions produced in turn an epidemic of leptospirosis in man Van der Hoeden[15] has comprehensively shown that the supposed *L geffeni* is no other than *L grippotyphosa*

Microtus may also be an occasional carrier of *L sejroe L batatiae L australis A* [79, 80] and of *L 'Sorex'* [80]

Micromys minutus soricinus is the habitual carrier of *L bataviae* in the rice fields of northern Italy Mino[81] has counted 1200 nests of these little rodents in 140 hectares (346 acres) of rice fields and among the animals examined he found that 21 9 per cent were carriers Unlike the other mouse species the *Micromys* does not live on the dykes but constructs its nests by hanging them on the stems of the rice plant As a consequence its urine falls directly into the semistagnant water of the rice field The *Micromys* is also a carrier of *L bataviae* in Finland [79]

Mus spicilegus is a frequent carrier of *L sejroe* and *L saxkoebing* and it may also harbor *L ballum*

Mus musculus is the principal carrier of *L ballum* This leptospire easily infects the white mouse and many strains of this animal are contaminated with this parasite thus constituting a danger for the experimental worker In fact laboratory cases of infection by *L ballum* are relatively numerous *It has been shown that the mouse may remain a carrier for more than 100* days after exper mental infection [65]

Mus musculus is also found to be one of the carriers of *L sejroe* [79, 80] and Parnas[80] have also isolated *L grippotyphosa* from it

L sejroe like the related *L saxkoebing* is most frequently spread by mice belonging to the genus *Apodemus* a genus which together with *Epimys brevicaudatus*, is also responsible for the dissemination of *L autumnalis* in the Far East and in Europe it may harbor *L pomona*[83] and *L batatiae* [81]

Arvicola terrestris has been recognized as a carrier of *L grippotyphosa*, *L saxkoebing* and *L Sorex* [80]

In all probability many other species of mice are carriers of leptospires especially in the tropical regions Since however in their case it is usually a question of single isolations of the parasites from the kidney it is not yet possible to confirm whether they are in fact true carriers or rather temporary shedders of leptospires

Among the insectivores note may be made of the genus *Sorex* the so called shrew mouse which at least in Central and Eastern Europe is the carrier of a leptospire corresponding to the type *Poi*

In the Union of Soviet Socialist Republics the hedgehog—both *Erinaceus europaeus* and *E auritus*—is a carrier of leptospires the systematic position of which is still badly defined [84] In Czechoslovakia Kmety[85] has isolated from the same species a leptospire similar to *L australis A* Parnas[80] has isolated *L batatiae* and *L Sorex* from *E reumanicus*

Finally it may be mentioned that in the Andaman Islands Collier and Esseveld[86] have isolated from the brain of a bat belonging to the genus *Cynopterus* a leptospire later classified by Collier and Mochtar[87] as *L schuffneri* The same type was also isolated later from the blood of a patient

In Java Collier and Mochtar [8, 9] have also isolated from the kidneys of bats belonging to the genus *Cynopterus* two strains of *Leptospira* belonging to a new serologic type *L cynopteri*

With regard to the possibility that bats may be carriers of pathogenic leptospires it is worth mentioning that in central Africa in the region between the rivers Sagan and Omo where Archetti and I[70] established the existence among the inhabitants of infections from leptospira the natives believe that the illness is spread by bats which are very abundant around the wells from which the men and animals drink The same belief is also reported by Rizzotti[81] who records the presence of cases of leptospirosis in the south of Ethiopia Rizzotti states that the Amhara in northwest Ethiopia call the illness *bescid elelithof* which means bat sickness

These opinions of natives merit a certain consideration it must not be forgotten that in Africa the Galla who inhabit British East Africa and Southern Ethiopia had noted correctly the relationship between malaria and the mosquito both of which they were already calling by the same name long before this connection was even suspected in Europe On the other hand I have searched unsuccessfully for the presence of leptospires in numerous bats captured in the rice growing regions of Spain[63]

Among domestic animals the dog undoubtedly has the major epidemiological importance for man The diffusion of leptospires among dogs is very high and their infection is obviously facilitated by their habit of sniffing where other dogs have urinated The leptospires that most often infect dogs are *L canicola* and *L icterohaemorrhagiae* The first is the species predominant in most European countries in America and in Australia *L icterohaemorrhagiae* on the other hand is more prevalent in Italy and Finland Alexander *et al*[4] hold that *L canicola* better adapted to the dog prevails in more developed countries where there is greater opportunity for contact between dog and dog while *L icterohaemorrhagiae* should be more frequent in less developed countries of predominantly agricultural economy where there is greater opportunity for contact between dogs and rodents This hypothesis is not easy to support In fact in Italy there is also a distinct predominance of *L icterohaemorrhagiae* in dogs that live in the large cities where contact with small rodents is less apt to occur It is possible however that *L canicola* predominates where the density of the canine population is higher and the contacts between the dogs are more frequent and easier

In nature the dog may also become infected with other types of leptospires namely *L grippotyphosa L sejroe L australis* A *L pomona L autumnalis L bataviae* leptospires of the *hebdomadis* group and with a special serotype isolated by Van Riel[97] in the Congo region of Africa However it is not known whether they may become carriers of such types

Dogs may fall ill with a serious form of leptospirosis and their mortality especially in young animals is very high Sometimes the leptospires provoke a chronic and progressive nephritis which causes the death of the animal after one or two years irrespective of whether it is a leptospire carrier

After infection by *L canicola* dogs often become carriers less often after infection by *L icterohaemorrhagiae* For example Klarenbeek[17] found leptospiruria in 59 per cent of dogs infected with *L canicola* and in only 26 per cent of those infected with *L icterohaemorrhagiae* Yamamoto[93] found respec

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Mus musculus is the principal carrier of *L. ballum*. This leptospire easily infects the white mouse, and many strains of this animal are contaminated with this parasite, thus constituting a danger for the experimental worker. In fact, laboratory cases of infection by *L. ballum* are relatively numerous. It has been shown that the mouse may remain a carrier for more than 100 days after experimental infection.[65]

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In Java, Collier and Mochtar[88, 89] have also isolated from the kidneys of bats belonging to the genus *Cynopterus* two strains of *Leptospira* belonging to a new serologic type, *L. cynopteri*.

that in young pigs *L pomona* may produce an evident illness that is sometimes quite serious and that in the pregnant sow may produce abortion

Pigs remain carriers of these two types of leptospire for a long period (one year according to Schmidt and Giovanella[16]) and sometimes they eliminate enormous quantities of leptospires with the urine Persons concerned with the feeding of pigs easily incur infections of leptospira These infections usually evince the symptoms of the so-called aseptic meningitis and they were known for a long time as young swineherds meningitis

The infected urine of the pigs via the drains of the sty may also pollute nearby water courses and ponds thus infecting those who by reason of their work or through bathing may come into contact with such waters[93 99]

An interesting example is described by Mitov Jankov and Savov[100] in Bulgaria where in 1953 there was a serious epidemic due to *L pomona* among the inhabitants of the Stalin (Varna) region following a flood that had submerged numerous pigsties existing in the locality

The pig is subject to infection by numerous other types of leptospire From the kidneys of pigs Kmety Plesko and Chvlo[6] have isolated *L canicola L sejroe* and *L ballum* Coghlan Norval and Seiler[101] have also isolated *L canicola* from the urine of the pig It is not known however whether the pig may actually become a carrier for these types or for what length of time

An interesting report by Kmety *et al*[6] states that pig carriers may be seronegative or else may possess antibodies for a type of leptospire different from that eliminated with the urine

Leptospirosis is also a serious problem in cattle and although it has been widely studied especially in the United States the Union of Soviet Socialist Republics and Israel there does not yet appear to be a satisfactory solution of it The cattle may fall sick with leptospirosis either in a mild form or to a serious or even fatal degree A high percentage of abortion in cows is attributed to leptospirosis especially in the United States where the Department of Agriculture has calculated that the annual financial loss caused by *Leptospira* infections in cattle has amounted to as much as $112 282 000[102]

I do not know nor shall I discuss the accuracy of this figure since it is irrelevant to my subject I emphasize merely that the serum of cattle very often shows an anti *Leptospira* agglutinin level that is rather difficult to interpret because it generally relates to healthy animals that have not had morbid manifestations even in the past Clearly finding anti *Leptospira* antibodies in a cow that has aborted does not justify one in unhesitatingly attributing responsibility for the abortion to the leptospire especially when it is pointed out that the presence of leptospires in the fetus or in stillborn calves is hardly ever proved In addition the experimental infection of pregnant cattle produces abortion much more rarely than many authors believe

Nevertheless it is undoubtedly true that cattle may become ill with leptospirosis and also may remain carriers and shedders of leptospires for long periods of time In the United States and in Australia the type of leptospire most often involved is *L pomona* in Israel and in North Africa it is *L grippotyphosa* and in the Soviet Union an *L bovis* that probably corresponds to

tively 1.9 per cent and 3.3 per cent. In the United States Alexander *et al.*[4] found 8 carriers among 92 dogs seropositive for *L. canicola* (8.7 per cent) but none among 32 that were seropositive for *L. icterohaemorrhagiae*.

The state of carrier is maintained in dogs for a considerable time (at least two to six months according to Brunner and Meyer [4]) as has been shown experimentally; it never persists, however, as long as it does in rats. It should also be noted that the emission of leptospires with the urine occurs in a discontinuous manner so that a single negative test is not sufficient to exclude the possibility that a dog is a carrier.

Generally, the percentage of dogs that become carriers after infection does not seem to be very high. Recently it fell to my colleagues and to me to examine a training school for Alsatian wolf dogs belonging to the police. Shortly before it had been possible to verify serologically cases of illness due to *L. canicola* that had occurred among the dogs. Sera from all the animals of the school were examined when the acute phase of the small epidemic had already passed. Forty-seven dogs, almost all of the 52 dogs examined, were clearly seropositive for *L. canicola*, often with very high titers of antibodies indicating recent infection. Among 43 of these dogs examined, 6, or 14 per cent showed the presence of *L. canicola* in the urine. This is a fairly high percentage, but not extremely so.

The high frequency of *Leptospira* infections in dogs contrasts with the rather low frequency of infections of canine origin in man. This is obviously due to the fact that man and his foodstuffs have less occasion to become contaminated with the urine of dogs than with that of the small rodents.

Besides dogs, the jackal is also a habitual carrier of *L. canicola*. Van der Hoeden[95] has attributed considerable importance to this animal in sustaining the epizootic of *L. canicola* which periodically affects cattle in the south of Israel. It is, however, probable that this importance is not so great. In fact, as Van der Hoeden himself admits,[96] the almost complete extermination of the jackals in the region has not noticeably influenced the progress of the epizootic; evidently the infection also spreads from bovine to bovine and from dog to dog.

The cat, theoretically greatly exposed to the infection because of its contact with mice and rats, is rarely identified as a carrier, however. In Indonesia cats have been found that are carriers of *L. bataviae* and *L. javanica*.[97] Klarenbeek[17] has observed cats in The Netherlands that are carriers of *L. canicola* and Gaub (unpublished data) in Alaska found cats that carried *L. hebdomadis*.

Silver foxes may also be carriers of pathogenic leptospires, whether of *L. icterohaemorrhagiae* or, in the Union of Soviet Socialist Republics, of two special types called DVA and DVB, which the author has found to correspond respectively to *L. hyos* and *L. pomona*. It is not known whether these animals remain carriers for a short or long time. In any case, their epidemiological importance for man is very limited.

Another domestic animal that is often a carrier of pathogenic leptospires and a source of human cases of infection is the pig. The two types of leptospire that most often infect the pig are *L. pomona* and *L. hyos*. Little is known of the morbid manifestations due to this second type. However, it is known

that in young pigs *L pomona* may produce an evident illness that is sometimes quite serious and that in the pregnant sow may produce abortion

Pigs remain carriers of these two types of leptospire for a long period (one year according to Schmidt and Giovanella[98]) and sometimes they eliminate enormous quantities of leptospires with the urine Persons concerned with the feeding of pigs easily incur infections of leptospirn These infections usually evince the symptoms of the so-called aseptic meningitis and they were known for a long time as young swineherds meningitis

The infected urine of the pigs via the drains of the sty may also pollute nearby water courses and ponds thus infecting those who by reason of their work or through bathing may come into contact with such waters[85 99]

An interesting example i described by Mitov Jankov and Savov[100] in Bulgaria where in 1953 there was a serious epidemic due to *L pomona* among the inhabitants of the Stalin (Varna) region following a flood that had submerged numerous pigsties existing in the locality

The pig is subject to infection by numerous other types of leptospire From the kidneys of pigs Kmety Plesko and Chylo[8] have isolated *L canicola* *L sejroe* and *L ballum* Coghlan Norval and Seiler[101] have also isolated *L canicola* from the urine of the pig It is not known however whether the pig may actually become a carrier for these types or for what length of time

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L grippotyphosa and an *L ictulina*, the systematic position of which is still uncertain have been described It may be noted that strains isolated from cattle in Israel appear to possess some serologic characteristics differing slightly from those of *L grippotyphosa* so that some authors maintain that it should be considered separately with the name *L bovis* In Israel, infections by *L canicola* are also quite frequent [97]

Cattle are able to shed leptospires in the urine only for limited periods of time According to Gillespie *et al* [3] such periods average about thirty days but in some cases may exceed one hundred days Bernkopf and Olitzki[56] in Israel have never succeeded in finding leptospires in the urine of seropositive cows It is to be noted however that these investigators were limited to a simple direct microscopical search for the spirochetes

The quantity of leptospires contained in the urine is very high even greater than 1 million per cc in a case reported by Gillespie *et al* [3] it reached 100 million per cc One can well understand how such enormous quantities of leptospires if coming into contact with water or moist soil may easily spread the contagion to both animals and man

Even so it seems that *L pomona* of bovine origin so rarely causes cases of infection in man that in the United States leptospirosis is essentially a veterinary problem and not one of human medicine

The case for *L grippotyphosa* is different in fact, both in Israel and in the Union of Soviet Socialist Republics numerous cases of human infection contracted by direct or indirect contact with bovine carriers have occurred The reason for this difference in infection has not yet been explained

Another still obscure problem is posed by this question why in the United States do cattle often present agglutinins for *L sejroe* when this leptospire in contrast to *L pomona* has never been isolated from them? Indeed in one case, Galton *et al* [103] isolated a strain of *L pomona* from the urine of a cow that was seropositive only for *L sejroe*

Similar observations have been made not only in cattle but also in man by Wiesmann[104] in Switzerland In my own researches on the other hand I have not observed that either human or bovine serum from subjects who have recovered from an infection by *L pomona* appears able to agglutinate *L sejroe* to any significant extent

Sheep and goats may also fall sick with leptospirosis sometimes in a very serious form Eighteen per cent of the mortality among lambs in New Zealand is due to *L pomona* according to Webster and Reynolds [105] as much as 44 per cent mortality among goats in Israel according to Van der Hoeden [106] is due to *L grippotyphosa* In Turkey Hakioglu[107] has found leptospires in the kidneys of sheep

Naturally infected sheep may void leptospires for at least nine months according to Webster and Reynolds [105] but Van der Hoeden has not found leptospires in the urine of convalescent sheep [106]

It must therefore be admitted that in Israel sheep in addition to transmitting the infection from one to another contract it from cattle with which they come into contact or with mice that are carrying *L grippotyphosa*

The horse is another animal that is very receptive to many types of lepto

spire In addition to becoming ill with the normal sort of infection due to leptospires the horse is also often subject to a particular form of iridocyclitis of relapsing development that often leads to blindness and is called in Italian *malattia della luna* (moon sickness)

Horses however insofar as is known to date do not seem to become true carriers They may eliminate leptospires only for a short time after the infection[108]

Pathogenic leptospires have occasionally been isolated from other animals However these have been isolated discoveries in animals that have little contact with man To these cases apart from any doubts as to whether they are true carriers one may attribute a negligible epidemiological significance The following is a list of these animals and the types of leptospire that they have been found to harbor *Citellus citellus* (*L pomona* and *L grippotyphosa*) *Cricetus cricetus* (*L grippotyphosa*) *Herpestes javanicus* (*L javanica*) *Fiber zibethicus* (*L sejroe*) *Apodemus sylvaticus* (*L sejroe* and *L Sorex*) *Neomys fodiens* (*L grippotyphosa*) *Castor canadensis* (*L icterohaemorrhagiae*) *Mustela kodiensis* (L *hebdomadis*) *Ondatra sp* (*L icterohaemorrhagiae*) *Clethrionomys rutilus* (*L icterohaemorrhagiae*) *Vulpes herimani* (*L hebdomadis*) *Nesokia bandicota* (*L australis* A *L pyrogenes* and *L icterohaemorrhagiae*) *Myocastor coypus* (*L icterohaemorrhagiae*) and *Evotomys glareolus* (*L grippotyphosa*)

Our discussion of man has been left to the last It has already been noted that man becomes a carrier and a source of infection only rarely In humans leptospires generally disappear from the urine within 40 days from the onset of the infection However Schueffner and Bohlander[109] have found leptospires in the urine of 2 convalescents for 45 days Carnier and Railly[110, 111] in 2 other cases for 100 and 103 days and Wimaers and Renoux[11] for 3 months Frugoni and Capellani[113] have described 2 cases in which healthy subjects who had been in contact with sick patients were eliminating leptospires with their urine Finally Manine Cristau and Plazy[114] Cristau[115] and Lettit[116] described 5 cases of interhuman infection in subjects who had assisted the sick during an epidemic that occurred in Lorient France in 1917 Cases of infection by sexual contact that occurred in Alaska[5] and one described by Doeleman[4] are also to be noted

The Identification of Carriers

The simplest way in which carriers may be identified is by the examination of a drop of urine or of the sediment obtained from it after high speed centrifugation in the dark field of the microscope When one is dealing with animals that may be sacrificed one may also make microscopic preparations of a pulp of the renal cortex Instead of direct examination in the dark field one may resort to examination of a preparation that is colored by a method of silver staining

Pulp of the kidney or the urine under examination may also be inoculated into the peritoneum of guinea pigs young chicks or hamsters (in searching for *L canicola*) Then the inoculated animal is kept under observation to see whether it may die show signs of sickness or produce anti *Leptospira* antibodies Better still a blood culture may be made in a suitable medium draw

ing blood from the animal six to seven days after the inoculation The culture may also be prepared with pieces of renal cortex obtained under sterile conditions

In my opinion the best method if the animal may be sacrificed is to carry out a direct culture from the kidney if on the other hand the animal must be kept alive then it is best to inoculate 2 to 3 cc of urine into the peritoneum of a guinea pig and to carry out a blood culture after 6 days The blood cultures must be examined after 7 15 and 30 days They should be discarded if still negative only after such a period

When a direct culture from the kidney is done care must be taken to seed only a very small fragment of tissue in each test tube so as to avoid introducing *into the culture any antibodies contained in such tissue* as these might inhibit the development of any leptospires that may be present

When carrying out inoculations of urine it must be remembered that quite often the leptospiruria is intermittent, consequently it is necessary to draw further samples of the urine at different times or on different days These samples must then be inoculated into the test animal immediately, as any delay might endanger the vitality of the leptospire

If the animal under examination voids acid urine it is advisable before drawing the sample to feed it on an alkaline diet or to give it some sodium bicarbonate or citrate

Disinfection of the Carriers

I do not intend to discuss here the various measures that it is expedient to adopt to protect subjects exposed to contagion spread by animal carriers this is a topic related to prophylaxis of the disease One thing that concerns the fight against animal carriers more closely is the need to distinguish the animals that are noxious or at least useless and may be killed such as small wild rodents from the useful ones which instead one may attempt to disinfect However disinfection of an animal carrier is not easy to accomplish Brunner and Meyer[117] have been particularly concerned with this topic They have established the fact that although penicillin is effective against acute infection by leptospira in the dog it is nevertheless incapable of disinfecting the animal in spite of the fact that it is found in high concentration in the urine

These investigators have achieved their best results with the use of streptomycin Doses of 40 mg / kg body weight administered 3 to 5 days in succession have always succeeded in achieving the desired purpose Aureomycin also gave some good results (5 to 40 mg kg) for 3 days

In any case the problem of carrier disinfection especially of dogs and the large domestic animals is one that would be worth studying deeply Thus far no such study has been undertaken

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LEPTOSPIROSIS INFECTIONS IN MAN

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Introduction Weil is credited with having first described cases of leptospirosis in humans as early as 1886[1] although Landouzy reported his own observations in 1883 The classic disease is characterized by fever hemorrhagic phenomena hepatic renal and vascular decompensation and a high fatality rate In 1915 Inada[2] isolated the etiologic agent a spirochete later identified as a leptospire by Noguchi[3]

Studies by various European Australian Japanese Indonesian and American investigators have identified many immunologically distinct leptospires as pathogenic for man and a host of other animals These specific microorganisms are naturally adapted to unique epidemiologic patterns and they produce highly variable clinical features in animals they infect The human manifestations are variable and it behooves physicians to recognize both the common and the unusual clinical features as well as the environmental hazards that lead to infection of man

The clinical manifestations of classic Weil s disease the milder forms of leptospirosis observed in a tropical environment and the North American types will be described

Classic Weil s disease (Leptospira icterohaemorrhagiae) Following an incubation of 4 to 19 days the disease begins abruptly often with a rigor as the first symptom Fever develops this continues between 102 and 104 F for about 8 days and falls by lysis The symptom complex that usually develops includes prostration severe and unrelenting headache anorexia and myalgia Muscle tenderness is a characteristic clinical feature and arthralgia may be intense Nausea vomiting and diarrhea are frequently present and occasionally severe Mental confusion and delirium may occur earlier in leptospirosis than in other infectious diseases

Injection of bulbar and palpebral conjunctivae a very common sign appears early and persists for several days Subconjunctival hemorrhages may develop but increased lacrimation is unusual Other ocular manifestations during the acute stage of illness include hemorrhagic lesions of the retina temporary and permanent oculomotor paresis optic neuritis and papilledema Optic atrophy is rare Leptospiral uveitis is a common complication noted from 2 weeks to 12 months following the onset Prognosis is good for complete recovery of normal motor and visual functions

Petechial lesions of the gastric and intestinal mucosa may lead to profuse bleeding from the gastrointestinal tract Cutaneous lesions may be petechiae or may resemble the exanthems observed in rubella or scarletina Severe

peripheral vascular decompensation comparable to that observed in the rickettsioses may contribute to a fatal outcome. Myocardial involvement may impair the circulatory status. Jaundice usually appears at the end of the first week simultaneously with defervescence. It may intensify rapidly and persist for a month or more. Deaths occur in jaundiced patients but the intensity of icterus is not necessarily of prognostic significance. Hepatomegaly develops in the jaundiced but splenomegaly is uncommon.

Renal decompensation is characteristic of Weil's disease. Moderate proteinuria may be noted when the illness is mild but in seriously ill patients proteinuria is often heavy. After the first week of disease oliguria or anuria may provoke severe azotemia and electrolyte imbalance of the lower nephron type. Fatalities often result from this serious complication. Diuresis is an obviously favorable omen and fortunately permanent renal damage is unusual.

Central nervous system signs may dominate the clinical picture. Nuchal rigidity can be marked. The spinal fluid pressure is sometimes elevated while microscopic examination reveals a lymphocytic pleocytosis. In jaundiced patients the cerebrospinal fluid may be yellow. Neuritis occasionally complicates the convalescence but usually meningeal signs subside completely.

The peripheral blood usually reveals a leukocytosis with a preponderance of neutrophils. A hypochromic anemia is frequently noted.

A death rate of approximately 30 per cent occurs in jaundiced patients who are severely ill. During the early stages of illness vascular collapse or hemorrhage from the gastrointestinal tract may cause death. Usually the patient succumbs with uremic signs during the second week of illness. The clinical manifestations that develop from derangement of the kidney, liver or vascular system are the most vital.

Mild cases. Milder forms of leptospirosis occur in Malaya, where this diagnosis was confirmed in 244 of 852 febrile illnesses investigated in 1954 and 1955.[4] Leptospires isolated from the blood in 90 cases were subsequently identified. These represented 12 of the 20 serogroups recognized in the classification proposed by Wolff and Broom.[18] Organisms of the *icterohaemorrhagiae* and *bataviae* serogroups are usually responsible for severe Weil's disease and were isolated from 6 and 10 cases, respectively, in this study. The remaining strains usually produce milder illness[5] and did so in Malaya, as evidenced by the low mortality rate of 0.8 per cent (TABLE 1).

The clinical manifestations presented by these patients consisted of an acute febrile phase lasting about eight days and a convalescent phase of approximately two weeks duration. Onset, abrupt in 70 per cent of cases, was often marked by rigor. Shortly thereafter fever, malaise, anorexia, headache, myalgia, and retro-orbital pain rapidly developed (FIGURE 1).

Anorexia and nausea occurred in 96 per cent of the patients. Eighty-five per cent of the cases experienced emesis at least once during the febrile period and many had almost continuous vomiting or retching. Mild diarrhea rarely occurred and, except for these few instances, bowel function was undisturbed. During the febrile phase headache and retro-orbital pain were continuous. Intensity of these symptoms varied in proportion to fever. Subjective myalgia

TABLE 1
SEROGROUPING OF LEPTOSPIRES OF HUMAN ORIGIN IN MALAYA 1954 AND 1955

Serogroup	Number of strains
pyrogenes	18
hebdomadis	10
bataviae	10
canicola	9
grippotyphosa	8
autumnalis	8
celledoni	8
icterohaemorrhagiae	6
schuffneri	5
australis A	4
javanica	2
pomona	1
djasiman	1
Total	90

CLINICAL MANIFESTATIONS OF LEPTOSPIROSIS
(182 military patients)
SYMPTOMS

GASTROINTESTINAL SYMPTOMS 96%
HEADACHE 95%
MYALGIA 77%
RESPIRATORY SYMPTOMS 71%
ABRUPT ONSET 70%
RETROBULBAR PAIN 55%
PHOTOPHOBIA 31%
ARTHRALGIA 29%

FIGURE 1. Salient subjective findings in military patients with leptospirosis. Reproduced by permission from *Transactions of the Association of American Physicians*.[18]

was generalized and most severe in the calf, abdominal, pectoral, and cervical muscle groups. Cough, present in half of the patients, was productive in 50 per cent of those with this complaint. Hemoptysis occurred in 60 per cent of the cases with a productive cough and was severe in two instances. A few patients complained of dyspnea, chest pain, coryza, and sore throat, but these did not constitute prominent features of the illness. Visual disturbances were never detected and photophobia was unusual (FIGURE 2).

Physical signs coincided with those usually observed in leptospirosis. Injection of the conjunctivae was present in 85 per cent of the cases, appearing within 24 hours after onset. Its intensity as graded by the physician varied from 1+ to 4+, the majority being graded 4+. Generalized lymphadenopathy was detected in one half of the patients but enlargement of the nodes was less than is ordinarily observed in infectious mononucleosis, scrub typhus

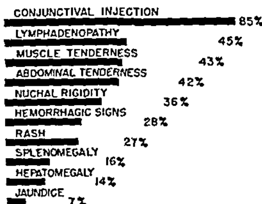


FIGURE 2 Objective clinical manifestations of leptospirosis in military patients. Reproduced by permission from *Transactions of the Association of American Physicians*[1]

or dengue Objective evidence of muscular tenderness was demonstrated in 43 per cent of the cases This finding created difficulty in evaluating the significance of pain particularly over the abdomen costovertebral angles and neck Appendectomy was performed unnecessarily in one instance Examination of spinal fluid was performed in ten patients who had marked stiffness of the neck Of these six revealed lymphocytic pleocytosis ranging from 40 to 400 cells per cu mm

Hemorrhagic signs appearing from the fourth to eighth days of disease included the following in decreasing order of frequency hemoptysis hematemesis epistaxis petechiae subconjunctival hemorrhages melena hematuria ecchymoses and submucosal hemorrhages in the pharynx Abnormal bleeding was observed in patients infected by all serogroups except *javanica pomona* and *sentoti* Bleeding was severe in two patients with hemoptysis one of whom died on the fourth day after onset *L australis B* was isolated from the fatal case Cough and signs of pulmonary congestion prompted roentgen examination of the chest in forty two cases Abnormalities varying from slightly increased vascularity to small localized areas of consolidation were revealed on the films in eleven cases Of these six presented physical signs and roentgen evidence of pneumonia In one instance these findings dominated the clinical course This patient s blood yielded *L grippotyphosa* in culture Other leptospires identified with clinical findings of pneumonia were from the *pyrogenes canicola* and *schuffneri* serogroups

Skin eruptions observed in one fourth of the group appeared between the fourth and eighth days of disease and persisted from a few hours to several days These lesions consisted of either small macules or maculopapules and

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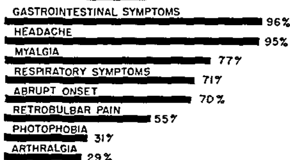


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tations cardiovascular renal and hepatic decompensation rarely occur A high incidence of aseptic type meningitis is characteristic in patients with swineherd s disease In Alabama Schaeffer[12] has reported the clinical features observed in 26 of 50 patients who contracted *L pomona* infection while swimming in a creek After an incubation period of 2 to 10 days the illness was characterized by chills and fever headache nausea and vomiting myalgia and meningismus Some had conjunctival injection and photophobia Diarrhea abdominal pain and *transient maculopapular eruptions were present in* a few cases Lymphocytic pleocytosis and slight elevations of spinal fluid pressures *were found in 6 patients who showed signs of meningitis* After an illness of 5 to 14 days all recovered uneventfully except for a few who experienced a brief uncomplicated relapse

Beeson and Hankey [13] in the United States and Broom [14] in England have incriminated leptospirosis as a cause of benign serous type meningitis Beeson demonstrated significant rises in titers of leptospiral antibodies in 4 per cent of 535 paired sera obtained from patients with meningitis of unknown etiology Therefore leptospirosis should be considered as a diagnostic possibility along with lymphocytic choriomeningitis mumps herpes poliomyelitis and Coxsackie and ECHO viruses as well as the various neurotropic encephalitides

L autumnalis was isolated first in Japan where the clinical illness was recognized as a mild febrile disorder of seven days duration occurring in harvesters during the autumn months [1] Icterus ocular complications and death occurred rarely

In 1943 an illness resembling dengue appeared in soldiers at Fort Bragg N C and was described by Daniels and Grennan [15] The cardinal features consisted of a short febrile period moderate prostration splenomegaly and a rash on the anterior aspects of the tibia After extensive studies by numerous investigators Tatlock[16] adapted the agent thought to be a virus to several laboratory animals and to fertile hens eggs The filtrable agent reproduced the characteristic manifestations in volunteers In 1951 Gochenour[3] identified the etiologic agent as a leptospire immunogenically similar to those of the *autumnalis* serogroup

Stroup and Riley[17] recently reported the occurrence of maculopapular lesions *over the pretibial area in two siblings presumably infected by L pomona* Each patient became ill eight days after swimming in a stagnant pond where pigs watered The onset was marked by fever anorexia and malaise headache lethargy and vomiting appeared within two days Examination on the third day of disease revealed mild muscular tenderness and nuchal rigidity Proteinuria microscopic hematuria and mononuclear pleocytosis were also demonstrated *On the fifth* day of disease conjunctival injection was noted and maculopapular lesions resembling erythema nodosum appeared over the pretibial area After forty eight hours the eruption faded completely Both patients recovered uneventfully after a total illness of about one week

Cultures for leptospires were negative Paired sera from both patients were *examined at the Walter Reed Army Institute of Research* Washington D C They revealed a marked rise in agglutination lysis antibody titers for *L pomona* Elevated titers for *L icterohaemorrhagiae* and *L autumnalis* were in

were distributed over the head and neck trunk, and upper extermities Lesions resembling the rose spots identified with typhoid fever were seen in a few patients on the upper extremities chest and abdomen

Splenomegaly and hepatomegaly were unusual occurring in only 15 per cent of the cases Icterus detected by inspection alone was rare

The total leukocyte count was usually normal but leukocytosis and leukopenia did occur Neutrophilia was noted in 80 per cent of the patients during the febrile period

Qualitative proteinuria casts and increased cellular elements were demonstrated at some time during the febrile phase in 80 per cent of leptospirosis patients Oliguria and mild azotemia developed in only 27 cases It never persisted longer than 3 days

Liver function was evaluated serially by means of the van den Bergh reaction total and fractional protein determination and Kunkel s flocculation test Liver function as evaluated by these tests was disturbed in two thirds of the patients Elevations of total bilirubin values appeared early and rapidly returned to normal with defervescence Abnormalities in the albumin globulin ratios and Kunkel s test developed at the beginning of convalescence and persisted for at least 2 weeks Clinically overt jaundice was observed in only 11 patients 2 of whom died Leptospires isolated from the 2 fatal cases were identified subsequently as *L bataviae* and *L australis B* Icterus was also observed in patients infected by organisms in the *hebdomadis canicola schüffneri* and *icterohaemorrhagiae* serogroups

Defervescence usually by rapid lysis marked the beginning of convalescence All symptoms and signs promptly subsided urinary findings returned to normal and icterus if present gradually cleared A few patients experienced a relapse characterized by a return of fever symptoms physical signs and proteinuria for two or three days during the second and third weeks after onset of the acute illness Convalescence was complicated once by thrombophlebitis and another time by unilateral atrophy of the quadriceps muscles One man suffered two attacks of leptospirosis separated by four months On these occasions cultures of blood yielded *L schüffneri* and *L bataviae* respectively

With these exceptions recovery was uncomplicated and patients resumed their normal activities in three to four weeks

North American leptospiral types Small localized outbreaks and sporadic cases of human leptospirosis occur in the United States The serogroups heretofore reported include *icterohaemorrhagiae canicola pomona*[6, 7] *bataviae* and *autumnalis*[9]

In the United States as elsewhere *L icterohaemorrhagiae* produces illness of variable severity Leptospires of the *bataviae* serogroup provoke clinical manifestations comparable to classic Weil s disease[6] *L canicola* produces slightly different clinical findings since jaundice seldom occurs and an aseptic type meningitis is common[11] In other respects *canicola* fever resembles Weil s disease

The leptospire *L pomona* which is responsible for swineherd s disease was isolated in Pomona Australia by Derrick[11] in 1942 The disorder is usually mild with a febrile course of from five to seven days Hemorrhagic manifes

LABORATORY DIAGNOSIS OF LEPTOSPIROSIS

By William S. Gochenour, Jr., Chester A. Gleiser, and Martha K. Ward
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The past decade has seen an increasing awareness of the prevalence of leptospirosis in man, in his domestic animals, and in wildlife in the United States. The public health and livestock sanitary problems posed by these infections and their economic significance to the livestock industry are being increasingly appreciated. This has resulted in an ever growing demand for diagnostic laboratory support in clinical diagnosis, epidemiological investigation, and research. It is the purpose of this paper to review and to evaluate the methods currently available to the leptospiral diagnostic laboratory.

It is perhaps belaboring the obvious to state that to meet the clinician's requirements adequately, the laboratory methods employed must be specific for leptospirosis and reproducible from time to time and in any laboratory, and must unequivocally confirm or negate the clinical impression of leptospirosis within a reasonable period of time. Retrospective diagnostic confirmation may well interest the epidemiologist, but it is of limited value to the clinician. From the practical standpoint, methods for widespread adoption must not be unduly expensive, laborious, or hazardous. Furthermore, they must be within the capabilities of all competent medical laboratory technicians.

Before discussing laboratory methods and techniques, it appears appropriate to review certain characteristics of leptospires and of leptospiral infections in man and in animals that are pertinent to the selection of specimens submitted for examination, their collection and handling, and the choice of the laboratory methods to be employed.

The pathogenic leptospires are fastidious organisms in their environmental and nutritional requirements. They are markedly susceptible to low *p*H, to the common disinfectants and antiseptics, to drying, and to ordinary pasteurization temperatures. *In vitro* they are sensitive to most antibiotics and are readily overgrown and destroyed by bacterial contaminants in culture media. In a neutral or slightly alkaline environment, however, they may survive for many months at ordinary temperatures, and they have withstood freezing for protracted periods.

In man and in animals, leptospiremia is associated with the initial febrile period of disease. At this same time leptospires may often be demonstrated in the cerebrospinal fluid. Leptospires are rarely demonstrable in the blood after the abatement of fever and the appearance of detectable circulating antibodies, usually in the second week of illness. *However, they may be found* for varying periods in the urine, in the kidney tubules, and on occasion in the anterior chamber of the eye.

Laboratory confirmation of the diagnosis of leptospirosis may be made by microscopic visualization of the organisms in appropriate body fluids, by histological demonstration of leptospires and their accompanying pathological changes in affected organs, by isolation of the organisms by direct culture or

terpreted by Gochenour to represent nonspecific cross immunological reactions Serum from another sibling who was exposed to the same stagnant pond, revealed antibody titers of 1 100 for *L pomona L icterohaemorrhagiae* and *L sentoti*

Summary and conclusion The clinical features of human leptospirosis vary considerably causing illnesses as severe as fulminant hepatitis and as mild as influenza The classic manifestations that suggest the diagnosis of Weil's disease are fever severe prostration icterus renal decompensation hemorrhagic phenomena and vascular collapse Milder forms of leptospirosis are more difficult to recognize but a leptospiral etiology should be considered in any febrile patient when severe headache vomiting myalgia conjunctival injection proteinuria and neutrophilia are noted Leptospirosis is to be suspected further whenever there is a history of exposure to those environmental conditions that favor infection

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as to be microscopically demonstrable within a few days after inoculation Chief among the disadvantages are the costs in laboratory space and personnel and the hazards and laboriousness of the methods One additional caution should be noted Many of the Swiss white mouse colonies of the United States are infected with *Leptospira ballum* This has caused delay and confusion in the investigation of the etiology of other diseases and must be excluded in instances where only animal isolation of leptospires has proved successful

Direct culture of patient s blood in the early febrile stage of disease has been employed for more than thirty years A number of media have been developed for the isolation and maintenance *in vitro* of these organisms In our experience Fletcher s semisolid medium[5] Schuffner s modification of Verwoort s medium[6] and Korthoff s medium[7] have been quite satisfactory Blood cultures have been made in the field and at the bedside by inoculation of bottles of medium closed with rubber skirted diaphragm stoppers at the time of venipuncture and in the laboratory from glass bead defibrinated blood Both methods were equally efficacious in yielding positive cultures One point of technique warrants special consideration In our experience it was essential to use minimal inocula (approximately 0 03 ml per culture tube or bottle) and to inoculate replicate tubes if positive cultures were to be obtained Larger quantities of the same specimens yielded negative results We attribute this to the presence of inhibitors in the specimen which if not diluted sufficiently prevented leptospiral multiplication That these inhibitors may be specific antibodies is suggested by the successful isolation of leptospires with this technique in 12 instances[8, 9] from blood specimens having a serum leptospiral agglutinating titer of 1 100 or greater

As leptospires grow slowly *in vitro* it was necessary to incubate cultures for a minimum of twenty eight days before they could safely be discarded as negative Of those positive, leptospires were demonstrable in one third by the seventh day of incubation in one half by the fourteenth day and in the remainder at the twenty eighth day The use of the semisolid medium greatly facilitated the periodic examinations required as these cultures could be examined macroscopically for the characteristic disk of leptospiral growth approximately 2 cm below the surface of the medium obviating in many instances tedious dark ground microscopic examination

In our experience the sole disadvantage of direct culture to t e demonstration of leptospires is its unsuitability for the examination of bacterially contaminated specimens With suitable specimens however our much greater success with direct culture than with animal inoculation in the isolation of leptospires from patient s blood leads us to agree with Fletcher[10] that for this purpose the use of laboratory animals offers no advantages over direct culture but merely adds to the burden of the diagnostic laboratory

Serologic confirmation of the diagnosis of leptospirosis rests upon demonstration of a significant (at least fourfold) rise in specific antibody in the examination of acute and convalescent sera While the presence of antibody reflects experience of the host with leptospires no single serologic positive finding regardless of the titer observed is adequate to substantiate unequivocally a diagnosis of acute leptospirosis This is particularly true of agglutinating

by animal inoculation or by demonstration of a significant rise in specific antibody through the examination of acute and convalescent sera These methods will be considered in terms of the requirements mentioned above

Microscopic demonstration of leptospires in blood, cerebrospinal fluid or urine by dark ground or phase contrast illumination affords the theoretical advantages of rapidity simplicity and economy In practice the low concentration of leptospires in these fluids affords small chance of their demonstration

Further, the ease with which artifacts such as protoplasmic extrusions from formed blood elements and fibrin tendrils moving by Brownian motion may be mistaken for leptospires severely limits the usefulness of the method Exceptions are the urine of swine and of rodent carriers which are often so heavily populated as to resemble well grown cultures With these exceptions we feel that microscopic examination of blood and other body fluids is worthless as a diagnostic laboratory procedure and we consider suspect any diagnoses resting solely on this method for confirmation

Histological demonstration of leptospires in the affected organs of individuals succumbing to the disease may be accomplished by the employment of silver impregnation techniques The technique of choice at the Armed Forces Institute of Pathology Washington D C is a modified Warthin Starry staining method[1] In arriving at a histopathological diagnosis of acute leptospirosis the pathologist must consider that leptospires unrelated to acute disease in an individual may be harbored in the kidney tubules This coincidental carrier state should not be imputed to have etiological significance in the case of disease under study Rather diagnosis must rest on both demonstration of leptospires and compatible and significant pathological changes A case in point was the first recognition of porcine leptospirosis in the United States While leptospires were recovered and many of the swine involved had evidence of leptospiral infection the fulminating and fatal disease seen in the herd was properly attributable to hog cholera and salmonellosis The leptospiral infection was coincidental and most probably pre existing

A wide variety of laboratory animals has been employed in the isolation of leptospires These include in addition to the guinea pig the golden hamster[3] the Swiss white mouse[4] the *Meriones*[5] the baby chick[6] and the embryonated hen s egg[7] With the exception of the latter these animals were originally advocated because of their susceptibility to lethal infection by one or more leptospiral serotypes Recognition of increasing numbers of benign *leptospiral serotypes* that produce little or no detectable clinical disease in these animals has resulted in their present use largely as accelerated living incubators and as filters for contaminated materials Animals are inoculated intraperitoneally with relatively large quantities of the specimen (1 to 5 ml) and peritoneal fluid heart blood or both are examined for the presence of leptospires microscopically and culturally every day for the first 4 to 6 days postinoculation The principal advantages of the inoculation of laboratory animals are that materials unsuited for direct cultural examination may be used that large volumes of a specimen may be inoculated into a single animal and that, frequently leptospires may multiply so rapidly in the living host

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lent correlation with this test was obtained with the live antigen microscopic agglutination test performed in the Division of Veterinary Medicine

While the above mentioned tests are highly serogroup specific one very important fact must be noted Paraspecific responses or cross reactions with heterologous serogroups frequently may be observed at levels higher than those of the homologous organism Particularly notorious in this regard are instances of proved *Leptospira icterohaemorrhagiae* infection wherein antibody levels against *L canicola* often exceed those against the homologous leptospire Thus while valuable presumptive evidence of the identity of a leptospire may be afforded by the host response elicited following infection definitive establishment of its identity can be effected only by its isolation and characterization In this connection the Division of Veterinary Medicine WRAIR has been designated by the World Health Organization of the United Nations with the concurrence of the Surgeon General United States Public Health Service and the Surgeon General United States Army as the Leptospiral Reference Laboratory for North America Definitive identification of leptospiral strains can be made by this laboratory It is strongly recommended that isolates made from man and animals be sent to it for identification and characterization

The complement fixation test of Randall Wetmore and Warner [14] employing sonically disrupted leptospires as antigens is the oldest of the genus specific serologic tests It has been used extensively in the laboratories of the Division of Veterinary Medicine WRAIR the United States Army Tropical Research Medical Laboratory San Juan Puerto Rico and in the field laboratory in Kuala Lampur Malaya In these laboratories excellent results were obtained [15] in the diagnosis of human leptospirosis Results in the examination of animal sera have been disappointing and further study of the factors responsible is indicated The advantages of this test are its simplicity of performance and the requirement for employment of only a limited number of antigens in the test battery Its major disadvantage in addition to that noted above is that being genus specific it affords no information as to the presumptive identity of the infecting leptospire

The hemagglutination test of Chang[16] employing erythrocyte sensitizing substances (ESS) and the hemolytic test of Cox[17] are both genus specific In the evaluation study previously mentioned [13] good correlation with the microscopic live antigen agglutination test was obtained in the examination of human sera Both tests however gave disappointing results in the examination of animal sera Results of continued evaluation studies of the hemolytic test[15] would indicate that it is as efficacious in the laboratory diagnosis of human leptospirosis as is the microscopic live antigen agglutination test Its advantages of simplicity requiring but a single antigen and of freedom from hazard recommended it to the laboratory concerned with diagnosis of human leptospirosis *per se* or as a screening procedure to be employed prior to examination of sera with either living or killed microscopic agglutinating antigens

In summary we feel that laboratory diagnosis of leptospirosis is now within the capabilities of any public health livestock sanitary or hospital laboratory

antibodies which may remain at high levels for many months following infection

The serologic tests employed in the laboratory diagnosis of leptospirosis include microscopic and macroscopic agglutination tests with living or dead leptospiral antigens, complement fixation tests employing intact leptospires sonically disrupted leptospires or leptospiral extracts or metabolites as antigens hemagglutination tests using erythrocyte sensitizing substance (ESS) and hemolytic tests using an antigen similar to the latter

The agglutination lysis test of Schuffner and Mochtar[11] one of the earliest leptospiral serologic tests has been and is today the most widely used and it is the serologic method against which all others have been evaluated This is a microscopic agglutination test in which living leptospires are employed as antigens As are all of the leptospiral agglutination tests it is highly serogroup specific This requires the employment of an adequate battery of antigens if antibody against an unknown leptospire is to be detected This battery may range from as many as two dozen antigens in a reference laboratory examining sera from the entire world to as few as eight in a diagnostic laboratory concerned only with specimens derived from man in the United States In addition to the above the hazard attendant on using living antigens the laboriousness of the method and the problems of standardizing antigen sensitivity[12] are seriously limiting factors Further grossly contaminated sera and those containing high concentrations of preservative cannot be examined

The microscopic agglutination test employing killed antigens has been used extensively in this country and abroad The use of killed antigen eliminates the hazards attendant on the agglutination lysis test and also permits the examination of sera too heavily contaminated to be examined by this technique When killed antigens are properly standardized and are periodically checked for sensitivity and absence of autoagglutination their use yields results essentially identical with those obtained with living antigens In collaborative studies we have examined aliquots of the same sera in the Special Bacteriology Laboratories Communicable Disease Center Chamblee Ga and in the Division of Veterinary Medicine Army Medical Service Graduate School (now Walter Reed Army Institute of Research) Walter Reed Army Medical Center Washington D C These studies reported in part by Ward *et al*[9] illustrate the virtual identity of results obtained by use of these two methods It should be noted however that there are minor differences in interserogroup cross reactions observed with living and with killed antigens and that any selection of the test battery of diagnostic antigens to be employed should take this factor into consideration

Macroscopic plate agglutinating antigens thus far developed have not yet been adequately evaluated to determine whether the shortcomings of lack of sensitivity lack of stability and difficulty of interpretation that characterized earlier macroscopic agglutinating antigens have been overcome

The capillary tube agglutination test of Stoenner has recently been evaluated in a collaborative study with the Division of Veterinary Medicine Walter Reed Army Institute of Research (WRAIR)[13] In Stoenner's laboratory excel

lent correlation with this test was obtained with the live antigen microscopic agglutination test performed in the Division of Veterinary Medicine

While the above mentioned tests are highly serogroup specific one very important fact must be noted Paraspecific responses or cross reactions with heterologous serogroups frequently may be observed at levels higher than those of the homologous organism Particularly notorious in this regard are instances of proved *Leptospira icterohaemorrhagiae* infection wherein antibody levels against *L canicola* often exceed those against the homologous leptospire Thus while valuable presumptive evidence of the identity of a leptospire may be afforded by the host response elicited following infection definitive establishment of its identity can be effected only by its isolation and characterization In this connection the Division of Veterinary Medicine WRAIR has been designated by the World Health Organization of the United Nations with the concurrence of the Surgeon General United States Public Health Service and the Surgeon General United States Army as the Leptospiral Reference Laboratory for North America Definitive identification of leptospiral strains can be made by this laboratory It is strongly recommended that isolates made from man and animals be sent to it for identification and characterization

The complement fixation test of Randall Wetmore and Warner[14] employing sonically disrupted leptospires as antigens is the oldest of the genus-specific serologic tests It has been used extensively in the laboratories of the Division of Veterinary Medicine WRAIR the United States Army Tropical Research Medical Laboratory San Juan Puerto Rico and in the field laboratory in Kuala Lampur Malaya In these laboratories excellent results were obtained[9, 15] in the diagnosis of human leptospirosis Results in the examination of animal sera have been disappointing and further study of the factors responsible is indicated The advantages of this test are its simplicity of performance and the requirement for employment of only a limited number of antigens in the test battery Its major disadvantage in addition to that noted above is that being genus specific it affords no information as to the presumptive identity of the infecting leptospire

The hemagglutination test of Chang[16] employing erythrocyte sensitizing substances (ESS) and the hemolytic test of Cox[17] are both genus pecific In the evaluation study previously mentioned[13] good correlation with the microscopic live antigen agglutination test was obtained in the examination of human sera Both tests however gave disappointing results in the examination of animal sera Results of continued evaluation studies of the hemolytic test[19] would indicate that it is as efficacious in the laboratory diagnosis of human leptospirosis as is the microscopic live antigen agglutination test Its advantages of simplicity requiring but a single antigen and of freedom from hazard recommended it to the laboratory concerned with diagnosis of human leptospirosis *per se* or as a screening procedure to be employed prior to examination of sera with either living or killed microscopic agglutinating antigens

In summary we feel that laboratory diagnosis of leptospirosis is now within the capabilities of any public health livestock sanitary or hospital laboratory

We feel that the success attainable by direct cultural examination of blood in the early febrile period of disease warrants inclusion of this procedure in laboratory studies of febrile illness of undetermined origin At this time serologic confirmation of the diagnosis of leptospirosis may best be made by microscopic agglutination employing living or killed antigens When antigens are available the hemolytic test of Cox may well supplement the agglutination tests or supplant them in small hospital laboratories

Existing knowledge of leptospiral antigen antibody reactions does not encourage hope of reducing significantly the time required for serologic confirmation of the diagnosis of leptospirosis More promise lies in the development of methods for the earlier demonstration of leptospires

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EPIDEMIOLOGICAL PATTERNS OF LEPTOSPIROSIS

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Introduction

For many years the leptospiroses were considered rare diseases, but it is now well established that they occur in humans and animals throughout the world and that they constitute a major problem in certain domestic animals.[1-6] More than forty years of extensive research has gradually unraveled a characteristic epidemiological pattern. For clear understanding of this pattern complete epizootiological data are essential. An adequate knowledge of the distribution of leptospires in potential wild and domestic animal carriers and the factors that influence the role of these carriers in the transmission of leptospiral infections to humans is of particular importance. As pointed out by Van der Hoeden,[7] the lack of sufficient information may result in misinterpretation of facts, but further knowledge will clarify the original picture.

The leptospiral infections are zoonoses, that is, diseases transmissible from animal to animal and from animals to humans.[8] Infection in humans is accidental and, with rare exceptions, it represents a dead end in the chain of transmission.[9]

Although rodents and certain domestic animals are considered to be the primary animal carriers, recent investigations have revealed a variety of hitherto unrecognized wild mammal hosts,[10-13] and current studies are detecting additional sylvatic leptospiral carriers. Some of the reported isolations of different leptospiral serotypes and their hosts are presented in TABLE 1. In the kidneys of these host animals the leptospires display an affinity for the renal cortex and may be found nesting in the lumina of the convoluted tubules. They may be shed in the urine for long periods. Each leptospiral serotype is believed usually to have a primary animal host for which it is particularly adapted; however, these serotypes will infect other animals as well. Furthermore, a single animal species may become infected with a number of different serotypes or even harbor two types at once.[14]

Leptospiral Serotypes Known to Occur in Humans or Animals in the United States

As research has progressed in centers located in widely separated areas of the world, the number of antigenically distinct serotypes of the genus *Leptospira* found to cause leptospirosis has increased. With this increase in recognized serotypes and the detection of an even broader host range, the epidemiology of the disease has become more involved. In 1954 Wolff and Broom[15] published a suggested classification system based on serologic analysis that included 34 leptospiral serotypes isolated from man and/or animals. During the relatively short period since this report appeared, at least 25 additional serotypes have been described in Australia,[16, 17] Africa,[18] Europe,[19] Malaya,[11] and the United States.[20]

Although the primary problem in the United States appears to be with

TABLE 1

REPORTED OCCURRENCE OF CERTAIN LEPTOSPIRAL SEROTYPES IN MAN AND IN ANIMAL HOSTS

Leptospira			Host																						
			Domestic							Wild or pet															
Group	Type	Man	Cat	Cattle	Dog	Goat	Horse	Sheep	Swine	Bat	Bandicoot	Fox	Guinea pig	Hamster	Hedgehog	Jackal	Mouse	Mongoose	Opossum	Raccoon	Rat	Shrew	Skunk	Vole	Wildcat
I	L. icterohaemorrhagiae	94		85	86				87			86	88				89	91			90				
II	L. javanica		92															92			86				
	L. poi	9															93				93	67		93	
III	L. sarmin	94																							
IV	L. schüffneri	86								86											11				
V	L. canicola	86		13	91				53							13					58				
VI	L. benjamin	94																							
VII	L. ballum	91							95			10					96		91	10	23				
VIII	L. pyrogenes	86																			86				
	L. australis B	16															67				64				
IX	L. cynopteri									86															
X	L. sentot	94																							
XI	L. autumnalis	86			97						11						96		28	28	86			98	
XII	L. djasiman	94																							
XIII	L. australis A	100			101										67		67			20	99				
XIV	L. pomona	102		99	101	77†	103	99	99								104		29	10	9		10	98	10
XV	L. grippotyphosa	86		7		4								98			67			10	11			7	
XVI	L. hebdomadis	105		106	101												86							94	
	L. medanensis	94			94																				
	L. wolffi	94																							
	L. sejroe	107			108				95								107								
	L. saxkoebing	109			94												109								
XVII	L. bataviae	86	92		101												9				94			98	
XVIII	L. semeranga																				94				
XIX	L. andaman	86																							
XX	L. mitis	110							111																
	L. hyos	112							112																

Numbers in table indicate reference.

† This culture died before identification could be completed, but serum from the goat agglutinated with L. pomona antigen in a 1:2560 dilution.

L icterohaemorrhagiae L canicola and *L pomona* seven other serotypes have been isolated and serologic findings suggest the presence of at least two others *L autumnalis* was identified after an eight year period as the cause of Fort Bragg fever[21] *L ballum* has been found frequently in rural house mice and opossums and occasionally in rats and laboratory mice[22, 23*] Serologic evidence has suggested the presence of *L bataviae* infection in humans[24] *L grippotyphosa* in humans[5] and cattle[26] and *L sejroe* infection in cattle[26, 27] Recent cultural studies[10] done at the Field Station of the Communicable Disease Center (CDC) Newton Ga on wild mammals trapped in southwest Georgia have yielded a variety of leptospiral serotypes in animals hitherto unknown as hosts including raccoons skunks and wildcats In addition isolations have been obtained from opossums and foxes

During a 6-month period ending in March 1956[10] 870 mammals including 14 species were trapped in different areas of 6 southwest Georgia counties and suspensions of the kidneys were cultured Leptospires were isolated from 44 (5 4 per cent) Of these 16 proved to be *L ballum* 14 were *L pomona* 2 were *L australis* A 2 were *L grippotyphosa* 2 proved to be a new serotype of the *mitis hyos* serogroup tentatively designated as *L bakeri*[20] and 2 belonged to the *hebdomadis* serogroup Two additional cultures were found to belong to the *mitis hyos* group but are not identical with *L mitis L hyos* or *L bakeri* Of the 4 remaining 2 were lost prior to serotyping and the identification of 2 has not been completed In this series opossums raccoons striped skunks foxes and wildcats were found to harbor 1 or more of these leptospiral serotypes These 5 species represented 79 per cent of the individuals examined None of the infected animals showed any clinical signs of leptospirosis and no gross kidney lesions were observed Only 4 of the 44 infected animals were determined to be less than 1 year old

The highest incidence of infection appeared in striped skunks with isolations from 18 (14 per cent) of 132 animals It is notable that 11 (61 per cent) of these cultures proved to be *L pomona* The areas studied represented 3 groups of predominant vegetative cover pine pine and hardwoods and pastures and cultivated fields There was no great difference among the vegetative types in incidence of infection of the animals cultured Continuation of these studies at the Newton Field Station has yielded 7 isolations of *L autumnalis* from raccoons trapped within a 2 mile area[23] The striped skunk opossum and raccoon continue to show the highest incidence of infection although occasional isolations have been obtained from the spotted skunk the gray fox and the wildcat

Although comparatively little attention has been directed toward the study of wild mammals elsewhere in the United States Roth[9] recently isolated *L pomona* from an opossum in Louisiana and Reilly[31] reported serologic evidence that suggested *L canicola* infection in raccoons in the state of New York

Current information regarding the occurrence of leptospiral serotypes in the United States is summarized in TABLE 2

* Recently 1 proved human case and 3 probable cases of leptospirosis caused by *L ballum* contracted from Swiss albino mice have been found at the Rocky Mountain Laboratory Hamilton Mont[30]

TABLE 2

LEPTOSPIRAE IN THE UNITED STATES

Serotype	Original isolate	Known wild hosts	Occurrence			
			Man	Dog	Cattle	Swine
L icterohaemorrhagiae	Japan Germany	Rat	Common	Occasional	?	?
L canicola	The Netherlands	Dog cattle	Occasional	Common	Rare	Rare
L pomona	Australia	Swine skunk raccoon wildcat opossum	Occasional	Rare	Common	Common
L autumnalis	Japan	Opossum raccoon	Rare	?	?	?
L ballum	Denmark	Mice gray fox opossum wild cat rat raccoon striped skunk	?	?	?	?
L grippotyphosa	Russia	Raccoon	Rare	?	Rare	?
L bataviae	Indonesia	*	Rare	?	?	?
L sejroe	Denmark	?	?	?	Sporadic	?
Hebdomadis serogroup (LT 11/)	United State	Opossum raccoon	?	?	?	?
L australis A	Australia	Raccoon opossum	?	?	?	?
L bakeri†	United States	Opossum	?	?	?	?
Mitis hyos serogroup (LT 81)	United States	Opossum raccoon	?	?	?	?

* Serologic evidence only
† *Mitis hyos* serogroup

Transmission

Of primary importance in the transmission of leptospiral infection to humans are the animal carriers that become urinary shedders of leptospires A diagram illustrating the current pattern of the transmission of leptospirosis in the United States is presented in FIGURE 1

After acute mild or more frequently inapparent infection the organisms become established in the kidney and are eliminated in the urine[1] Infections of humans and animals result from direct or indirect contact with the infected urine of these shedders For example direct contact may occur when individuals care for sick animals or handle the tissues of infected animals in abattoirs Indirect contact occurs when water or moist soil is contaminated and individuals are subsequently exposed while working or swimming or otherwise come in contact with the contaminated environment[3]

Arthropod vectors have not been incriminated as yet in the transmission of leptospirosis in nature[14] However Schlossberger and Langbein[33] were able to infect the tick *Ornithodoros moubata* by feeding it on guinea pigs infected with *L icterohaemorrhagiae* Transmission to normal guinea pigs was effected when the ticks were fed 31 days after infection These observations were confirmed by Burgdorfer and Pickens[34] Later Burgdorfer[35, 36] found that *Ornithodoros turicata* and another tick *Dermacentor andersoni*

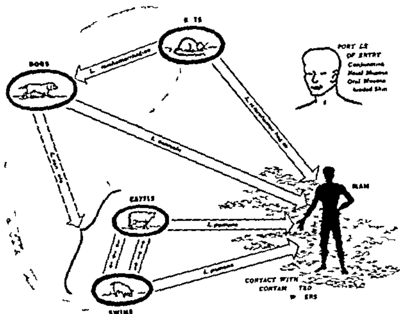


FIGURE 1 The epidemiology of leptospirosis in the United States This chart was modified by G W Fischer from Byrne[32] Reproduced by permission from the December 1955 issue of *Public Health Reports* official journal of the Public Health Service United States Department of Health Education and Welfare

became infected after engorgement upon *L pomona* infected hamsters and transmitted the disease to normal animals More recently *L grippotyphosa* was isolated from the European tick *Dermacentor marginatus S* from cattle in the Union of Soviet Socialist Republics[37] Isolated cases of leptospirosis had occurred among farm animals in the area where the ticks were collected Thus the detection of natural infection increases the possibility that arthropods are vectors

While leptospires may be shed in milk during the acute phase of the disease in lactating animals[38] Kirschner[39] found that the organisms survive only a few hours in whole milk that is leptospirocidal In milk diluted 1 20 or higher with neutral water they survive a number of days[40] There have been no proved cases of leptospirosis that could be traced to drinking infected milk

The portals of entry of infection are usually the nasal oral and conjunctival mucous membranes and abraded skin There is considerable doubt that leptospires penetrate the intact skin and it is unlikely that the digestive tract is an important portal of entry since the *pH* of the stomach is usually such that the organism may be destroyed quickly

Environments that favor the survival and spread of leptospires are wet soil stagnant water or slow moving streams that are neutral or slightly alkaline when temperatures are 22° C or above Under such conditions leptospires may survive for several weeks[41-44]

TABLE 3

REPORTED OUTBREAKS OF LEPTOSPIROSIS IN THE UNITED STATE

Date of onset	Location	No. of cases	Age range	Probable source	Serotype implicated
August 1940	Wrens, Ga.	35	Avg 16	Cattle	*L. pomona*†
July and August 1942 to 1944	Fort Bragg, N. C.	40/yr	Young adults (soldiers)	?	*L. autumnalis*
August 1942	Jackson Hole, Wyo.	24	15 to 19	Dogs	*L. canicola*†
August 1947	Calvary, Ga.	10	13 to 24	Dead mule in creek	?
August 1949	Swainsboro, Ga.	12	Young adults	Cattle	*L. pomona*†
July 1950	Geneva, Ala.	50	Adolescent and young adults	Swine	*L. pomona*†
July 1952	Columbus, Ga.	26	5 to 20 and 2 adults	Swine, dogs, cattle	*L. canicola*

With the possible exception of the outbreak at Fort Bragg, all outbreaks were attributed to swimming in contaminated water.
† Serologic evidence only.

Outbreaks and Sporadic Cases of Leptospirosis in Humans in the United States

It is not surprising that large outbreaks may occur after group swimming in contaminated water. Several such outbreaks have been reported in the United States, as shown in TABLE 3. The first of these occurred in August 1940 near Wrens, Ga.[45, 46] and involved 35 patients who had been swimming in Brushy Creek. Clinical signs included fever, nausea, vomiting, headache, some diarrhea, and a rash on the pretibial areas of the legs. It was found that approximately 100 yards upstream from the swimming area offal from slaughtered cattle had been dumped by the local butchers. This unusual disease was given the name of Brushy Creek or pretibial fever. A similar outbreak of pretibial fever occurred among troops at Fort Bragg in August 1942, and again in the summers of 1943 and 1944.[1, 47, 49]

All patients involved in the outbreaks were quartered in the same area of the post. Many, but not all, had been swimming in the nearby ponds, so the source of their infection remains obscure. Not until 1950 was Fort Bragg fever proved to be a leptospiral infection caused by a member of the *L. autumnalis* group.[21] After this discovery, McCroan reinvestigated the Brushy Creek outbreak and 2 other similar undiagnosed epidemics that had occurred in Georgia, one* in the late summer of 1947 near Calvary[46] and the other during the same season in 1949 on the Ohoopee River near Swainsboro.[46] Antibodies to *L. pomona* were demonstrated in serum samples collected at the time of the outbreak and during the subsequent investigation in 1953 from several patients involved in the Brushy Creek and Ohoopee River outbreaks.

A follow-up investigation in 1953 of another epidemic of an unknown disease that had occurred in August 1942 in two small communities in Wyoming and Idaho revealed serologic evidence of leptospiral etiology.[50] Here again all

* Investigated by Elmer Hill and John E. McCroan of the Georgia State Health Department, Atlanta, Ga.

persons affected had been swimming in the same pool located at Jackson Hole Wyo to which dogs and other animals had access

In late July 1950 an outbreak occurred in Geneva Ala[51] involving about 50 persons who became ill with an influenzalike disease after swimming in a creek in which dead swine had been seen floating above the swimming hole Serologic evidence indicated that *L pomona* was the cause

When in late July 1952 an outbreak of illness occurred in a small community near Columbus Ga involving 26 persons who had been swimming in a dammed up creek leptospirosis was suspected immediately[5] The subsequent investigation of this outbreak including laboratory studies[53] presented an excellent picture of many of the principles involved in leptospiral epidemiology *L canicola* infections thought for many years to be limited to dogs and man were demonstrated in swine cattle and dogs that had access to the creek All persons who became ill had been swimming in the creek The data showed a significant correlation between attack rates and the frequency and extent of exposure to the water Attack rates were consistently higher among those giving a history of diving and immersing their heads

With the possible exception of the Fort Bragg outbreaks each of these water borne leptospiral epidemics followed a similar pattern They occurred in the late summer during drought periods there was presumed contamination of a stagnant pond or slow moving creek by urine from infected animals and transmission to humans by immersion in the contaminated water Such water borne outbreaks following swimming or accidental immersion have been reported in many other areas of the world[8, 54-60]

Of even greater importance in the epidemiology of leptospiral infections are the opportunities for exposure encountered by workers in certain occupations particularly abattoir workers cane and rice field workers dairy farmers animal husbandrymen veterinarians sewer workers and trench diggers For example in Italy Spain the Far East and Pacific regions where the rice fields are flooded and worked by barefoot laborers leptospirosis is an important health and economic problem[9, 61-63] The fields usually abound with mice and rats Babudieri[9] found in a survey of 509 apparently healthy workers in Italy that 20 per cent had leptospiral antibodies The percentage of infection increased in different groups as the length of service increased until it reached 80 per cent in those who had worked in the rice fields for 20 years or more Approximately 66 per cent of these infections were due to *L bataviae* A similar situation exists in Australia among cane field workers Derrick and his coworkers[64] found that 57 per cent of the known cases of leptospirosis in North Queensland Australia occurred among cane field workers and were attributed primarily to infection with *L australis* A although 10 other leptospiral serotypes were incriminated No cases of leptospirosis have been encountered among cane and rice field workers in the United States Swineherd s disease due to *L pomona* and *L mitis* infections is common in certain parts of Europe among butchers abattoir workers and others who have had contact with swine[65-67] Kirschner[68] reported that 67 per cent of 130 cases in New Zealand occurred among dairy farmers and the remainder in pig breeders or

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When in late July 1952 an outbreak of illness occurred in a small community near Columbus Ga involving 26 persons who had been swimming in a dammed up creek leptospirosis was suspected immediately[5] The subsequent investigation of this outbreak including laboratory studies[53] presented an excellent picture of many of the principles involved in leptospiral epidemiology *L canicola* infections thought for many years to be limited to dogs and man were demonstrated in swine cattle and dogs that had access to the creek All persons who became ill had been swimming in the creek The data showed a significant correlation between attack rates and the frequency and extent of exposure to the water Attack rates were consistently higher among those giving a history of diving and immersing their heads

With the possible exception of the Fort Bragg outbreaks each of these water borne leptospiral epidemics followed a similar pattern They occurred in the late summer during drought periods there was presumed contamination of a stagnant pond or slow moving creek by urine from infected animals and transmission to humans by immersion in the contaminated water Such water borne outbreaks following swimming or accidental immersion have been reported in many other areas of the world[8, 54-60]

Of even greater importance in the epidemiology of leptospiral infections are the opportunities for exposure encountered by workers in certain occupations particularly abattoir workers cane and rice field workers dairy farmers animal husbandrymen veterinarians sewer workers and trench diggers For example in Italy Spain the Far East and Pacific regions where the rice fields are flooded and worked by barefoot laborers leptospirosis is an important health and economic problem[9, 61, 63] The fields usually abound with mice and rats Babudieri[9] found in a survey of 509 apparently healthy workers in Italy that 20 per cent had leptospiral antibodies The percentage of infection increased in different groups as the length of service increased until it reached 80 per cent in those who had worked in the rice fields for 20 years or more Approximately 66 per cent of these infections were due to *L bataviae* A similar situation exists in Australia among cane field workers Derrick and his co workers[64] found that 57 per cent of the known cases of leptospirosis in North Queensland Australia occurred among cane field workers and were attributed primarily to infection with *L australis* A although 10 other leptospiral sero types were incriminated No cases of leptospirosis have been encountered among cane and rice field workers in the United States Swineherd s disease due to *L pomona* and *L mitis* infections is common in certain parts of Europe among butchers abattoir workers and others who have had contact with swine[65, 67] Kirschner[9] reported that 67 per cent of 130 cases in New Zealand occurred among dairy farmers and the remainder in pig breeders or

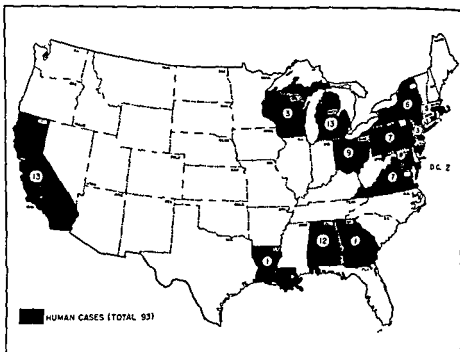


FIGURE 2 The geographical distribution of cases of Weil's disease in the period from 1905 to 1941[70]

abattoir workers The apparently lower incidence in the United States of human infection due to occupational exposure may be attributed to higher living standards and to mechanization of industrial and agricultural operations However the recent comment of Kalz[69] that the number of recognized and reported cases particularly in the United States and in Australia appears to be related directly to the location of interested workers and to the availability of laboratory facilities warrants some thought In recent years there has been an increase in reported cases in the United States The geographic distribution of known cases was summarized by Larson in 1941[70] and again in 1948 by Molner Meyer and Raskin[71] as shown in FIGURES 2 and 3 In the first period summarized which covered 36 years there were 93 cases in 14 states Seven years later the total number of cases had increased to 299 in 24 states In contrast a summary of cases detected through serologic tests in the CDC Laboratories during a 4-year period (1953 to 1956) plus those reported to the CDC by state health departments or to the National Office of Vital Statistics Washington D C during this time revealed a total of 392 in 39 states In addition 96 cases occurred in outbreaks reported between 1951 and 1956 The distribution of these cases is shown in FIGURE 4

Sporadic cases[72-75] are reported in the United States in veterinarians live stock men abattoir workers sewer workers dog owners and others Haunz and Cardy[76] recently reported 9 cases in a single family that were attributed to

FIGURE 3. The geographical distribution of human leptospirosis in the period from 1905 to 1948.[71]

infection from the family dog. During the past 3 years information has been obtained regarding the occurrence of 94 cases of leptospirosis in 27 states. Awareness of these cases came primarily from serologically positive sera referred to the Diagnostic Serology Unit, CDC Laboratories, Chamblee, Ga.,* and from state health departments. Subsequent inquiry to the state epidemiologists usually resulted in a history of the case. Others were reported by the National Office of Vital Statistics or were investigated on request by the CDC Leptospira Research Laboratory staff. Information concerning the probable source of infection was obtained on 64 of the 94 cases. Of these, 25 (39 per cent) had had contact with infected cattle or swine either in abattoirs or on farms; 17 (26 per cent) had been drinking, swimming, or had been accidentally immersed in presumably contaminated water; 12 (19 per cent) had had contact with dogs in their homes or in veterinary hospitals; 7 (11 per cent) had been exposed to rats; 2 (3 per cent) to wild animals; and 1 (2 per cent) to a goat.[77] The months of onset for these 94 cases presented in FIGURE 5 show the highest incidence during August and September. There were 84 males and 10 females in the group. While cases occurred in patients from 2 to 64 years of age, the peak appeared between the ages of 30 and 39 as shown in FIGURE 6.

* Appreciation is expressed to Joseph Schubert for data obtained from his laboratory.

FIGURE 4 The occurrence of human leptospirosis in the period from 1953 to 1956 The e 392 cases were reported through the Laboratory and Statistic Section Communicable Di ease Center Public Health Service Chamblee Ga the National Office of Vital Statistics Wash ngton D C and state health departments The figures in parentheses which total 94 epresent cases in published reports of epidemics from 1951 to 1956

FIGURE 5 The seasonal distribution of 94 cases of human leptospirosis in the United States from August 19 4 to August 195

The fact that the probable source of more than one third of these recent cases was found to be contact with infected cattle or swine may be attributed in part to the rapid spread of bovine leptospirosis in the United States since 1951, as shown in FIGURES 7 and 8 In 1954 the Agricultural Research Service of the United States Department of Agriculture[8] estimated that annual losses

from bovine leptospirosis were over 112 million dollars or 25 million dollars greater than losses from bovine brucellosis.

In large part the prevention and control of leptospirosis are aspects of the prevention and control of other diseases. Certainly the application of strict sanitary practices will play an important role. Protection of drinking water supplies for domestic animals from contamination, adequate drainage of wet muddy farm areas, and rat control are particularly valuable measures. Several

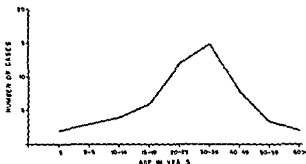


FIGURE 6. The age distribution of 55 cases of human leptospirosis in the United States from August 1954 to August 1957.

FIGURE 7. The reported occurrence of bovine leptospirosis in the United States during 1951.* Reproduced by permission of *The Proceedings of the United States Live Stock Sanitary Association.*

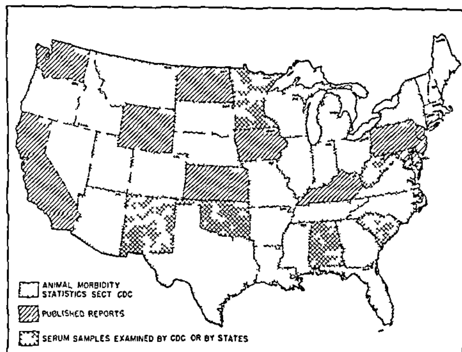


FIGURE 8 The reported occurrence of bovine leptospirosis during 1956

leptospiral vaccines are available for use[79, 81] in cattle and while results have been encouraging further evaluation of their effectiveness is needed

In nature leptospirosis has a wide host spectrum that yields a variety of serotypes As yet *L pomona* is the only serotype used in the preparation of vaccines for cattle While most infections in cattle and swine are caused by this serotype *L canicola* was isolated recently from a sick calf in Alabama[8] and serologic findings suggest the presence of *L sejroe*[6] and *L grippotyphosa* infections[26] Thus the use of a polyvalent vaccine should be considered The same consideration should be given to canine vaccines which at present are prepared only from *L canicola* and *L icterohaemorrhagiae* strains

Prevention and control of the disease in humans particularly in those exposed to it in their occupations depends largely upon improvement of working conditions Results of vaccination studies have been excellent in the Italian and Spanish rice field workers[8] among whom the incidence of infection is high The feasibility of similar immunization of abattoir workers veterinarians and others at risk should be studied

Discussion

The leptospiroses spread to humans under favorable environmental circumstances following contact with the tissues or urine of infected animals or contact with water or wet soil contaminated by animal carrier Higher living standards greatly influence the lower incidence in the general population

in the United States and other countries that report the disease in animals but have few human cases In the comparatively few occupations in which the problem is encountered better sanitary practices rat control and protective clothing can play an important role in reducing the hazard Outbreaks of the disease related to recreational accidents may be expected to occur sporadically

The detection of leptospiral serotypes hitherto unrecognized in the United States emphasizes the need for a more concerted effort by medical and veterinary laboratories to search for them As laboratory techniques for the isolation of leptospires are improved and applied more serotypes will be obtained from humans and from domestic animals Further study of the new wild mammal hosts to determine the incidence of infection in other parts of the country is indicated In addition little is known of the duration of the carrier state in these hosts Application of the recently developed bladder tap technique[83] to secure urine for direct culture would provide some of the information needed Future research in this direction will throw more light upon the role of these wild mammal hosts in the over all picture of leptospiral epidemiology and epizootiology

There has been much speculation as to the reasons for the apparent increase in incidence of leptospiral infections It may be accounted for in part by a greater awareness of the disease Current knowledge strongly suggests the existence of an epizootic or panzootic of the leptospiroses that may flow and ebb for some decades Until we have further knowledge of the conditions that favor the prevalence of leptospirosis we have no better explanation than Sydenham s theory of the *genius epidemicus* that contagious diseases are influenced by cosmic or atmospheric conditions

Summary

Leptospiral infections are known to occur in wild and domestic animals throughout the civilized world More than forty years of extensive research have revealed a characteristic epidemiogical pattern All leptospires known to infect animals are potentially pathogenic for humans While some serotypes appear to exhibit a certain host preference all of the more common types have been found to infect more than one animal species During the past two years investigations have detected in a variety of wild mammal hosts the presence of 5 leptospiral serotypes hitherto unrecognized in the United States

Of primary importance in the chain of transmission are the animal carriers of leptospires After acute mild or even inapparent infections the organisms *localize in the kidney and are shed in the urine for varying periods* These carriers serve as foci of infection for other animals and man

In the United States reported outbreaks of leptospirosis have been associated primarily with swimming in stagnant pools or creeks presumably contaminated by carrier animals Sporadic cases that occur are more frequently associated with occupational exposures among veterinarians abattoir workers sewer laborers dog owners and others exposed to a contaminated environment

Information concerning 94 sporadic human cases in the United States is presented These outbreaks and sporadic cases are discussed and compared with the epidemiology of leptospiral infections in other countries These facts emphasize the need for further research to clarify the significance of the new sylvatic hosts in the over all pattern of leptospirosis

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have infected salivary glands been found without concurrent infection in the central nervous system, which indicates an inherent inability of this species to transmit the disease as a symptomless carrier The transmitting potential of foxes however is great as shown by the fact that daily testing of experi

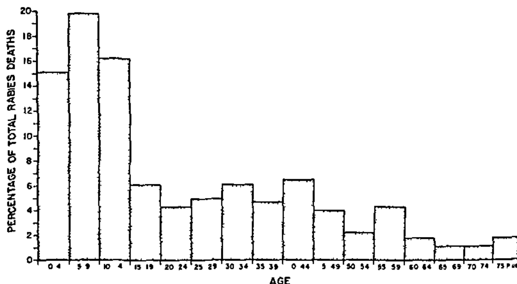


FIGURE 1 Percentage of human rabies deaths in the United States by age groups from 1944 through 1954

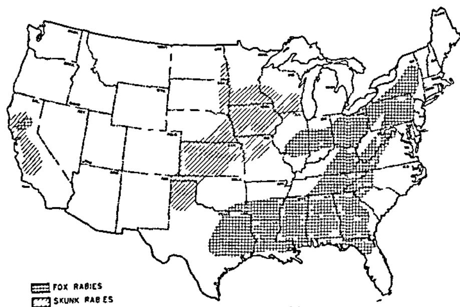


FIGURE 2 Principal sylvatic rabies areas of the United States

mentally infected foxes showed that their saliva contained rabies virus for long periods of time, in one individual for 17 days.

The most recent development in the problem of sylvatic rabies is the finding of rabies in insectivorous bats in the United States. This series of events began with the first isolation of rabies virus from bats in June 1953, when a yellow bat *Dasypterus floridanus* attacked a child near Tampa, Fla. This episode aroused great interest, prompting surveys and surveillance activities throughout the country. Since the first reported case in Florida, 175 cases have been diagnosed from 18 states in widely diverse geographical areas of the United States, 2 from 1 province of Canada, and 2 from Yugoslavia. Four species of tree living or solitary bats and 10 species of colonial or cave-dwelling bats have been implicated thus far. All are of the insectivorous variety. The greatest number of rabies virus isolations has been made from the Mexican free tailed bat *Tadarida mexicana* in the southwestern United States.

One of the many problems in the epizootiological puzzle of rabies in insectivorous bats is the question of asymptomatic transmission of the disease. It is well established that their chiropteran cousins, the vampire bats in Latin America, are capable in some instances of transmitting rabies for long periods of time without showing signs of illness. Some have suggested that this symbiotic host virus relationship may be present in the insectivorous bats. During current studies conducted by the Communicable Disease Center in a cave in the southwestern United States, four infected bats have been found in a random sample of 300 normal bats collected in flight. Rabies virus was recovered from the salivary glands, and not from the central nervous system, of these four bats, which remained clinically normal until sacrificed for testing. The ultimate test for asymptomatic carriers, however, that of demonstrating a continuous yield of virus from saliva of normal insectivorous bats observed over long periods of time, has not yet been done.

Although it has been possible to find the infection in normal bats of the aforementioned cave colony in frequencies ranging from 0.5 to 1.5 per cent, the rate of virus isolations went up to 5.1 per cent in dead and moribund bats collected in the midst of a die-off that lasted about three weeks. During this die off many of the ill bats exhibited convulsions and spasmodic contractions of the abdominal muscles. Vicious biting of one bat by another was observed in one instance. Monthly sampling for serum neutralizing antibodies in this cave revealed positive antibody rates which increased gradually from 14.7 per cent in the early part of the season to 28.2 per cent late in the season just before the colony left on its southward migration.

Investigations are now being carried out to discover the epidemiological significance of the bat rabies findings. Although as yet there has been no direct and definitive evidence of the natural transmission of rabies from insectivorous bats to man or to lower animals, the public health implications of such findings cannot be minimized. There are now well-documented reports of 35 episodes of bats proven to be rabid that had bitten human beings (about half were unprovoked attacks) and 2 human rabies deaths are attributed to exposure from rabid bats on an anamnestic basis since neither bat was recovered.

A few observations from the early phases of the current investigations may be of interest here It has been possible *experimentally* to infect by peripheral routes several species of animals including bats with virus isolated from the brains and salivary glands of naturally infected bats but in most instances this has been done with difficulty (Stamm *et al* 1956 Enright *et al*, 1955 Burns *et al*, 1956) This phase of the problem including attempts at simulated natural transmission, is described elsewhere in this monograph

The ultimate solution to the rabies problem is predicated on the control and eventual elimination of the disease from animal populations This may be accomplished by the establishment of transmission barriers such as animal immunization, elimination of stray dogs and the reduction of excessive numbers of wildlife vectors Perhaps the greatest advance in the control of rabies in the last decade has been the improvement of animal rabies vaccines and the successful use of canine vaccination The progress made in this field is discussed elsewhere in this monograph

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Discussion of the Paper

OSCAR SUSSMAN (*Bureau of Veterinary Public Health State of New Jersey Department of Health Trenton N J*) It has been contended by some observers when referring to the decrease of animal rabies in the United States during the period from 1944 through 1954 that this decrease has been associated with the use of new live culture avianized rabies virus vaccines Inference has been made that there is a direct relationship between these reductions and the new live culture vaccines The decrease shown was indeed substantial however it included reductions in areas where avianized vaccine was not used To attribute this reduction solely to the introduction of a new vaccine is naive and at least a loose use of statistics If there is any principle in epidemiological analysis it is that the secular movement of any disease is the resultant of many factors (multiple causation) and not merely the behavior of any single variable

I agree with Ernest Tierkel that vaccination is an excellent procedure around which to conduct a rabies control program I agree further that the avianized vaccine where properly administered to dogs is an efficacious prophylactic agent In determining the amount of credit however that can be attributed to the new live culture vaccines in the decrease of animal rabies I suggest that we examine the behavior of the disease in earlier times both for the United States as a whole and for particular geographical subdivisions with particular

reference to those periods when only phenolized vaccine was available for use and in which it actually was used

(1) In 1937 and 1938 11,500 animals were reported rabid in the United States In 1942 this number decreased to 7165 animals—a decrease of almost 38 per cent

(2) In the east south-central part of the United States in 1936 2250 animals were reported rabid and by 1939 only 745 were reported rabid—a decrease of 67 per cent

(3) In the Pacific Coast area 2129 animals were reported rabid in 1938 This figure decreased to 515 in 1940—a reduction of 75 per cent

(4) In the state of New Jersey in 1939 679 cases of rabies were reported This was reduced to 42 cases in 1943—a decrease of approximately 94 per cent

It is true that in the United States as a whole reported rabies increased from 7165 for the calendar year 1942 to 10 870 for the calendar year 1946 and then fell erratically to 5844 for the calendar year 1955 In the east south central portion of the United States the number of reported cases increased from 745 in 1939 to 2066 by 1945 dropped to 1476 by 1951 only to increase to 2719 by 1953 and then to fall to 973 by 1955 On the Pacific Coast reported rabies decreased from 2129 in 1938 to 515 in 1940 only to increase to 916 by 1944 then to drop to 75 by 1951 and then to increase to 425 by 1955

All of this demonstrates that reported rabies behaves in a cyclic fashion If one were to choose any particular interval of time in a particular geographical area a case could be made out for the use of killed vaccine for the control of stray dogs or for no effort whatsoever

It must be admitted in all fairness that Ernest Tierkel s citation of the changing nature of the rabies problem from one in which dogs are the predominant species involved to one in which wildlife seems to be the essential factor in the maintenance of the disease is good supporting evidence that vaccination of pet animals is an excellent prophylactic procedure I believe however that to ignore other possible factors such as increased awareness on the part of dog owners of their responsibility in disciplining their animals or in ignoring the ordinary behavior of the disease is to be unaware of the subtle interplay of the multiple variables in the natural history of any disease

It has been said that An epidemiologist is a disease investigator who slides into glory on the downward slope of an epidemic curve It appears that attempts have been made to slide the new avianized rabies vaccines to glory on the downward slope of a nationwide cyclic epidemic rabies curve

Avianized vaccine was discussed at the meeting of the American Public Health Association in New York N Y in 1949 A statement was made that in Staten Island N Y where there had been a considerable amount of rabies the previous year no cases of rabies were reported following the use of avianized vaccine in a field trial The audience was led to believe that this was due to the use of the avianized rabies vaccine This impression was not corrected until Morris Greenberg of the New York City Department of Health indicated that the safety of the product might be evaluated in this respect but that one could not validly claim that avianized vaccine was responsible for the observed

phenomenon Greenberg pointed out that *while the avianized vaccine was used in Staten Island where there had been rabies and now there was not any the avianized vaccine had not been used in Manhattan where there had been rabies and now there was not any*'

Phenolized vaccine has been used in many situations throughout the United States, and used successfully Certainly the previous United States and regional figures referred to in which definite decreases in incidence were observed could not be attributed to the avianized vaccine when it is remembered that in this period only phenolized vaccines were available These decreases were no less startling than those that occurred following the use of the avianized product in other situations

Different causal relationships other than the use of vaccines are actually involved in the decrease of rabies incidence This is particularly true in the case of dog rabies where proper control a firmer conviction of owner responsibility in supervising the activities of the dogs and the elimination of stray dogs results in a decrease of susceptible populations to attack by wild and predatory infected animals On the basis of all the evidence available I maintain that the use of any good vaccine aids in reducing the number of susceptibles among owned vaccinated dogs I have yet to see a stray dog jump on a table and ask to be vaccinated

In order to show conclusively that avianized vaccine is responsible for the reduction in reported rabies the following evidence must be submitted

(1) Secular trend lines in rabies prevalence maintain a negative slope

(2) If the foregoing statement is true then it must be demonstrated that (a) an increasing proportion of dogs vaccinated had been treated with avianized vaccine as compared to other vaccines and (b) that there was no statistical difference in the results between the groups of vaccinated dogs all other factors being equal

Merlin Kaeberle in his paper Newer Tools for the Prevention of Rabies in Domestic Animals pays deserved attention to the duration of immunity experiment conducted jointly by the United States Public Health Service Washington D C and Lederle Laboratories Pearl River N Y However he failed to mention that the phenolized vaccine and the avianized vaccine both protected 100 per cent of all dogs challenged 24 months after vaccination

I should like to raise several issues that appear to me to be of paramount importance in future rabies control procedures and in determining products to be used to control the disease in domestic animals

(1) *Recall immunity* Do multiple doses of phenolized vaccine administered at yearly intervals produce a more solid immunity than does the single dose of avianized vaccine given every 3 or 4 years? We have some evidence in our work with poliomyelitis and more conclusive evidence in Harald Johnson's[1] work on the phenolized product to the effect that multiple doses are of value in increasing total immunity The 39 month study of the United States Public Health Service comparing phenolized and avianized vaccines failed to take into account the annual vaccination system in determining the relative value of multiple doses of killed products and method of administration

(2) *Shelf life* Consideration must be given to the shelf life and to the

practitioner's handling of any product Live-culture vaccines when reconstituted require meticulous handling and may not be mishandled even when desiccated Their entire value depends on the introduction of sufficient live virus to cause infection and subsequent immunity This does not imply that phenolized products may not be abused however phenolized material can stand more abuse before its potency is lost than can avianized material

(3) *Safety versus species* Live vaccines must be used only in those species for which they have been proved safe Therefore if there is likelihood of utilization in unauthorized species a product of greater safety must be used even if some degree of immunity is lost The use of the avianized product in cats puppies cattle skunks and foxes has resulted in cases of atypical rabies

(4) *Sensitivity versus neurological manifestations* Sensitivity to egg protein may become a hazard when distemper products and other similar vaccines all are made from egg embryo tissue As to neurological manifestations following the use of phenolized killed products over 300 000 dogs in the state of New Jersey have been vaccinated over the last 5 years with no definite evidence of neurological manifestations

(5) *Live rabies virus in nonendemic areas* Aside from the benefit of recall immunity when annual vaccinations are made rabies control administrators are faced with the problem of the use of live rabies virus in areas where no cases exist Should the disease reappear following live vaccine usage the subsequent public reaction must be taken into account This consideration may appear inconsequential and a blot on the progress of scientific advancement but it is a definite problem in areas where the disease has been eliminated

(6) *Psychological value of annual vaccination* The psychological impact when annual vaccinations are held must be considered While in some areas this may be onerous in others it is considered good procedure for it not only aids in controlling rabies but it prescribes at least one time in a year when an owner must look closely at his pet and may help to prevent transfer of other animal diseases to the owner

In conclusion it is very difficult for one who has had considerable success in the use of the phenolized vaccine to see it buried so rashly before it is really dead Psychologically there is some reason for having an annual vaccination of dogs as has been proved by field experience among those of us who have administered these programs on a continuous basis Both vaccines certainly are valuable adjuncts in the prevention of rabies The downward slope of the epidemic curve in the last decade cannot however be attributed solely to the introduction of the newer avianized vaccine As one administrator and epidemiologist involved in the control of rabies in a state that has successfully eliminated the disease by the use of dog control phenolized vaccine and the elimination of stray dogs I cannot accept the definition of an epidemiologist or of a superior vaccine as one that merely slides to glory on the downward slope of an epidemic curve

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BAT RABIES EXPERIMENTAL HOST TRANSMISSION STUDIES

By Kenneth F Burns Dorothy Fresenius Shelton and Earl W Grogan
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The recovery of rabies virus from bats living under natural conditions in the United States indicates the need for evaluating the public health significance of these animals as reservoirs and carriers of this disease Are rabies infected bats purely of academic interest or do they constitute a true public health hazard? Is it likely that human subjects will be affected directly by the bite of such a bat except as a rare accident, or is it more logical to believe that bats may come to represent an additional sylvatic reservoir in this country contributing to a marked increase of the disease in animals and indirectly in man? These are the more important questions with which public health personnel are concerned

Originally the recovery of rabies virus from bats infected in nature was thought to be a unique single event localized within a small geographical area in Florida[1] Within 6 months however other rabies isolations were made from bats in Pennsylvania and Texas[3, 4] and thereafter from other states in the Southwest[5] the Midwest and the West[6] From 4 species of solitary and 9 species of colonial bats approximately 175 isolations of rabies virus have been made since June 1953 and 30 of these isolations have been associated with human exposures[7] Further circumstantial evidence concerned with 2 human rabies deaths in Texas would tend to incriminate bats as the vector of these 2 infections[8, 9]

In attempting to evaluate the public health significance of bat rabies we studied the ability of laboratory infected and naturally infected indigenous bats to transmit the disease to other animals under conditions of induced association in the laboratory

Further studies were conducted with other animals to determine their susceptibility to bat rabies viruses and in an attempt to determine whether these agents have assumed some of the characteristics ascribed to the phenomenon of fixation

MATERIALS AND PROCEDURES

Animals

Bats Tadarida mexicana (Mexican free tailed bats) used in this experiment were obtained from two sources Bracken Cave Texas and Carlsbad Caverns N Mex The group of 400 bats netted at Bracken Cave appeared physically normal at the time of capture and were maintained in captivity for a 21 day period prior to the initiation of the experiment The group from Carlsbad consisted of 50 encephalitic bats collected from the floor of the caverns during

an epizootic of rabies among Molossidae of the cave during August and September 1956 *

Twenty five *Antrozous pallidus pallidus* (pallid bats) were collected and shipped to us by Bryan P Glass of Oklahoma Agricultural and Mechanical College Stillwater Okla

Monkeys Five young adult rhesus (*Macaca rhesus*) monkeys were employed The animals weighed between 12 and 20 lb and had been imported directly from India by the United States Air Force They had not been used for any previous studies having been maintained at this laboratory for 5 months prior to the beginning of this experiment

Mice Three to four week-old Swiss albino mice weighing 8 to 10 gm were used throughout the experiment as indicators of infection for virus titrations and for the intracerebral mouse neutralization tests for reidentification of agents employed and serum antibody titrations

Other animals Fifteen guinea pigs 16 hamsters 19 rabbits 4 Spanish goats 6 chickens and 1 raccoon were included in the study for biting and induced-confinement experiments and for host susceptibility studies

Virus Strains

Six rabies strains isolated from the brains of *T mexicana* bats were employed in these studies The strains were identified as the virus of rabies by the intracerebral mouse neutralization test In addition to the bat isolates rabies fixed virus (Challenge Virus Standard) obtained from Lederle Laboratories Pearl River N Y was used for control purposes

Isolate No 2915 (third mouse-brain passage) was obtained from a pool of the brains of 2 of the naturally infected Carlsbad Caverns bats collected during the August 1956 rabies epizootic

Isolate No 2084 (fifth mouse brain passage) was obtained from a moribund bat collected on the floor of Carlsbad Caverns in September 1955

Isolates No 1063 (second mouse brain passage) and No 1248 (third mouse brain passage) were isolated from individual paralyzed bats collected in the San Antonio Texas area in July and December 1954

Isolate No 1009 13 (fifth mouse brain passage) was obtained from a pool of the brains of 5 apparently normal bats (asymptomatic) collected in Uvalde Texas in July 1954

Isolate No 768 (third mouse brain passage) was obtained from an individual bat exhibiting encephalitic manifestations at Fort Sam Houston Texas in April 1954

Diagnostic Criteria and Identification of Rabies Virus

Throughout the studies rabies virus was considered isolated either when Negri bodies could be demonstrated in brain smears from mice that died following a characteristic course of symptoms or when in the absence of these

* The cooperation of D G Constantine of the Veterinary Public Health Section of the Communicable Disease Center Public Health Service Atlanta Ga, is gratefully acknowledged

identification of the agent could be made by the standard neutralization test. This latter method, outlined by the Neurotropic Virus Disease Commission (since renamed the Commission on Viral Infections, Armed Forces Epidemiological Board), Walter Reed Army Institute of Research, Washington, D. C., as stated by Paul [10] incorporates equal volumes of undiluted inactivated rabies antiserum (horse) and serial tenfold dilutions of virus-infected mouse brains.

Rabies Virus Titrations

With all experimental hosts, each of the virus strains was titrated by the *intracerebral inoculation of tenfold dilutions into 8- to 10-gm., 3- to 4-week-old* white Swiss mice. The total dose inoculated is expressed as mouse intracerebral LD_{50}s (MIC LD_{50}).

Bacterial Sterility Studies

Bacterial sterility studies were employed throughout the experiments on all injectable materials using trypticase soy and thioglycollate culture mediums. After incubation at 37° C. and reading at 72 hours, all tubes having no clouding or turbidity indicated sterile inocula.

Collection of Saliva Samples

Collections of saliva samples were made by repeated swabbing of the mouths of the bats with 6-inch cotton-tipped applicators soaked in 1.0 ml. of 10 per cent rabbit serum saline containing 1000 units of penicillin and 2.0 mg. of streptomycin. The excess was expressed against the vial wall and, after 1 hour of incubation at refrigerator temperature (4° C.) with the antibiotics for control of bacterial contamination, the mixture was inoculated intracerebrally into mice.

Inoculations

Intracerebral inoculations of mice (0.03 ml.), bats (0.02 ml.), chicks (0.05 ml.), hamsters (0.05 ml.), guinea pigs (0.10 ml.), and rabbits (0.25 ml.) were made using a 27 gauge needle and a 0.25 ml. syringe employing sterile technique. In a similar manner, but with increased amounts, goats and monkeys received an intracerebral dose of 0.5 ml. of inoculum, using a 1.0-ml. syringe with a 22 gauge needle while the animals were under general anesthesia.

Intramuscular inoculations (gluteals) of hamsters (0.05 ml.), guinea pigs (0.10 ml.), rabbits (0.75 ml.) and a raccoon (1.0 ml.) were made using a 1.0-ml. syringe and a 22 guage needle. Bats received 0.02 ml. into their pectoral muscles with a 0.25 ml. syringe and 27 gauge needle.

Subcutaneous inoculation of bats (0.02 ml.) and rabbits (1.0 ml.) was made in the suprascapular area with 27 gauge needles and with 0.25 ml. syringes and 1.0-ml. syringes, respectively.

Intraperitoneal inoculations of guinea pigs (1.0 ml.) and hamsters (0.5 ml.) were accomplished with a 1.0-ml. syringe and 22 gauge needle.

Serum Collections

Serum specimens were obtained from the monkeys for assay of neutralizing antibody for rabies virus prior to and four months after confinement with bats and again when the challenged animals were moribund with rabies Sera of all other species of animals including fowl were collected just prior to exposure and either when moribund with clinical rabies or at time of sacrifice of those that remained asymptomatic

Serum Neutralization Tests

Serum neutralization tests were conducted according to the method quoted by Taul[18] A log neutralization index of 1 7 or greater was considered positive

INDUCED ASSOCIATION EXPERIMENT INVOLVING INFECTED BATS (*T mexicana*) AND NORMAL MONKEYS

Separate dog metabolism cages were utilized to confine a number of bats (*T mexicana*) and 1 monkey each Three of the cages each contained 100 bats inoculated with 32 000 MIC LD$_{50}$s of bat rabies isolate No 2084 intra cerebrally subcutaneously and intramuscularly respectively One cage contained the 50 naturally infected bats collected from Carlsbad Caverns One cage contained 100 normal bats as controls The cages were altered to consist of 2 compartments upper and lower separated by a metal grid containing openings large enough to permit passage of the bats The monkeys were caged in the lower compartments and the bats were placed in the upper compartments at the beginning of the experiment

Results

Early migration of the inoculated bats from the upper compartments of the dog metabolism cages to the lower compartments occupied by the monkeys was observed The bats did not appear to return to their own compartments This was believed to be due to the development of infection from the prodromal period in which the bats were irritable aggressive and restless to a state of fatal progressive paralysis No such picture was noted among the asymptomatic bats confined for control purposes Numerous altercations occurred resulting in bites inflicted on the monkeys by the infected bats The monkeys attempts to dismember the confined bats resulted in their being bitten on the fingers In addition to bite wounds it appears reasonable to assume that the monkeys received stab wounds in the gums and throat while consuming portions of the bat carcasses Many fragments of bats were discarded from each of the cages

Bats inoculated intracerebrally placed with monkey No 1 Of 100 intracerebrally inoculated bats all were dead by the eighteenth postinoculation day Fifty three bats that were removed between the sixth and eighteenth postinoculation days were examined for rabies infection the remaining carcasses were discarded due to mutilation by the monkey These 53 bats were autopsied and tissues were prepared in 13 pools each of brain and salivary gland Rabies

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TABLE 1

BAT RABIES EXPERIMENTAL HOST STUDIES

Intracerebral Challenge of Monkeys and Inoculation of Goats

Animal species	Bat rabies virus strain	MIC‡ LD	Animal No.	Death / inoculated	Postinoculation day	Virus recovery: Brain	Virus recovery: Salivary gland	Remarks
	2084 (Fifth mouse brain passage)	$10^{3.0}$	1	1/1	8	Pos	Neg	Monkeys challenged 120 days after exposure to laboratory infected rabid bats
			685	1/1	8	Pos	Neg	
			595	1/1	10	Pos	Neg	
Monkey (*Macaca rhesus*)	2915 (Third mouse brain passage)	$10^{4.7}$	2	1/1	8	Pos	Neg	Monkey challenged 120 days after exposure to naturally infected rabid bats
	Control CVS	10	697	1/1	7	Pos	Neg	Monkey challenged 120 days after exposure to asymptomatic bats
	2084 (Fifth mouse brain passage)	10	1	1/1	12	Pos	Neg	Encephalitis
Goat (*Capra* sp)	1248 (Third mouse brain passage)	10^{5}	2	1/1	11	Pos	Neg	Encephalitis
			3	1/1	13	Pos	Neg	
	1063 (Third mouse brain passage)	10 [illegible]	4	0/1	50	Neg	Neg	Repeated IC challenge with 1063 (MIC‡LD 10^{3} *) on 18th postinoculation day Negative S N I †

* Asymptomatic sacrifice
† Serum neutralization index
‡ Mouse intracerebral

isolated from the group of naturally infected bats with which this monkey had been in contact Monkey 697 whose contact had been with normal bats received 100 000 MIC LD_{50} s of challenge virus standard (CVS) The results are shown in TABLE 1

A prodromal period was noted in the monkeys as early as the fifth day for the CVS challenged animal and on the sixth to eighth days for those challenged with the bat rabies strains Fulminating infections developed with weakness of the extremities grinding of teeth ease of incitement to convulsive state moments of mental alertness subsequently drifting into subconsciousness followed by an ascending type paralysis Deaths occurred on the seventh to tenth postinoculation days

All monkeys were bled in the moribund state and sera from these bleedings were found to be negative for neutralizing antibodies to rabies Mice inoculated with brain tissue of all monkeys exhibited positive evidence of rabies while none of the monkey salivary gland tissues yielded a viral agent Histopathological examination of the monkeys brains revealed a meningoencephalitis including glial satellitosis perivascular cuffing and neuronophagia Sellers stained impressions and Stovall Black stained sections failed to reveal Negri bodies

virus was recovered and identified from all brain pools and all but 1 salivary gland pool the negative pool being composed of salivary glands of dead bats collected on the sixth postinoculation day

Bats inoculated subcutaneously placed with monkey No 685 Of the 100 subcutaneously inoculated bats 88 were removed dead between the sixth and twenty second postinoculation days Only fragments of the remaining 12 bats could be identified Twenty two pools each of bat brain and salivary gland tissues were prepared and inoculated intracerebrally into mice Five of the 44 pools yielded rabies virus 3 positive brain pools from bats dying the ninth seventeenth and twenty second postinoculation days and 2 positive salivary gland pools from bats dying the fifteenth and twenty second postinoculation days

Bats inoculated intramuscularly placed with monkey No 595 Of the 100 intramuscularly inoculated bats 59 dead were recovered between the sixth and twenty third postinoculation days the remaining 41 bats having been dismembered beyond recognition by the monkey Isolation materials contained in 16 brain and 16 salivary gland pools resulted in recovery of the virus in 5 instances from both brain and salivary glands of bats dying the sixth and thirteenth postinoculation days and from the brain only of bats dying on the twentieth postinoculation day

Bats infected naturally placed with monkey No 2 The 50 naturally infected *T mexicana* were caged with rhesus monkey No 2 Twenty four hours later 11 bats had died and were removed from the cage Six of the remaining sick bats died on the second postexposure day 3 on the third and 2 on the fourth From the 2 bats dying the fourth postexposure day 2 isolates were obtained (No 2915 Br and No 2915 SG) from pooled tissues of both brain and salivary gland tissue and these were identified as the virus of rabies Twenty eight bats were either eaten or mutilated and could not be utilized

Normal controls placed with monkey No 697 One hundred normal bats were placed with a fifth monkey On the twenty third postexposure day 48 bats were sacrificed for testing and from their tissues 13 brain pools and an equal number of salivary gland pools were prepared Intracerebrally inoculated mice yielded negative results Fifty two bats were mutilated beyond salvage or cannibalized by the monkey

None of the monkeys exhibited clinical symptoms of rabies or other disease during a 120-day period of observation no demonstrable neutralizing antibodies were observed in sera drawn prior to or 120 days after exposure

Challenge

Since all of the monkeys remained asymptomatic during the observation period each was challenged with rabies virus to prove its susceptibility to bat rabies virus

Monkeys Nos 1 685 and 595 were challenged intracerebrally with 6300 MIC LD_{50}s of strain 2084 four months after their contact exposure with bats experimentally infected with that strain Simultaneously monkey No 2 was challenged intracerebrally with 50 000 MIC LD_{50}s of strain 2915 which was

neuronophagia perivascular infiltration glial nodules and Negri inclusion bodies

Strain 1063 was inoculated (approximately 500 000 MIC LD_{50}s) into goat No 4 On the twelfth postinoculation day the animal refused water and food but after a 24 hr period it once again started to eat and drink and assume normal habits On the eighteenth postinoculation day the goat was challenged intracerebrally with approximately 8000 MIC LD_{50}s of bat rabies virus strain 1063 The animal exhibited no central nervous system symptoms during a 50-day observation period and was sacrificed Serum specimens collected at time of inoculation and 41 and 50 days postinoculation were negative for neutralizing antibodies for rabies The mechanism of immunity observed in this animal manifested by its resistance to challenge in the absence of discernible neutralizing antibody cannot be explained

Rabbits

Nineteen rabbits were utilized in experimental infection studies via intracerebral (I C) intramuscular (I M) and subcutaneous (S C) routes of inoculation resulting in infection and subsequent death of 13 of the group (TABLE 2) Six animals remained asymptomatic and were sacrificed after a 33 to 61 day observation period All 4 rabbits inoculated intracerebrally and 5 of the 7 inoculated intramuscularly died of rabies the mean incubation period for each of these routes being 17 days The 2 intramuscularly inoculated survivors were sacrificed on the thirty fifth and sixty first postinoculation days Of the 8 rabbits inoculated subcutaneously 4 became infected with rabies with a mean incubation period of 22 5 days and 4 of this group remained asymptomatic 3 being sacrificed after 33 days and 1 on the fortieth day

Rabies virus was recovered from the brains of 11 of the 13 rabbits succumbing to infection with bat rabies virus No viral agent could be recovered from

TABLE 2
BAT RABIES TRANSMISSION STUDIES IN RABBITS

Bat rabies strain	Route	Dose mouse LD_{50}	Deaths/ inoculated	Postinoculation day of terminal	Negri body	Virus recovery brain pos/No tested	Neutralizing antibodies pos/No tested
2084	I C	10	2/2	13 14	Negative	2/2	2/2
(Fifth mouse	S C	10	2/4	23 26 (33) (40)	Negative	1/4	3/4
brain passage)	I M	10 [illegible]	4/5	14 16 16 23 (61)	Negative	3/5	4/4
1009–13	I C	10^{4}	1/1	24	Positive	1/1	1/1
(Fifth mouse	S C	10 [illegible]	0/2	(33) (33)	Negative	0/2	1/2
brain passage)	I M	10	1/1	18	Positive	1/1	1/1
1248	I C	10^{5}	1/1	18	Negative	1/1	ND
(Third mouse	S C	10	2/2	20 21	Negative	2/2	2/2
brain passage)	I M	10^{5}	0/1	(35)	Negative	0/1	ND

Symbol ( ) all animals terminated after 30 days had remained asymptomatic for the observation period recorded ND test not performed

Induced-Biting Experiments Using Laboratory Infected *A. pallidus pallidus*

Fifteen asymptomatic *A. pallidus pallidus* were each inoculated intracerebrally with approximately 20,000 MIC LD_{50}'s of bat rabies strain 2084. The bats had been maintained at the laboratory for 11 days prior to inoculation. One bat dying on the second postinoculation day was discarded. Of the remaining 14 mammals, deaths occurred between the eighth and twenty-seventh postinoculation days, 7 of the group dying between the eighth and twelfth days. A period of 24 to 48 hours of marked irritability preceded the deaths. *Rabies virus was recovered from the brains of 13 of 14 bats, the single failure* being the brain of one of the 4 bats dying on the twelfth postinoculation day. Attempts to isolate rabies virus from the salivary glands were accomplished on 6 of the inoculated bats. Three of these yielded rabies virus. Pooled mouth swabbings collected 7 days postinoculation from 3 bats exhibiting symptoms of a central nervous system infection yielded rabies virus.

Six of the infected bats (including the three that yielded rabies virus from salivary gland) on the tenth, eleventh, twelfth, and fourteenth postinoculation days were induced to inflict multiple bites on the ears and abdomens of 6 guinea pigs. The guinea pigs survived a 60 day observation period with no clinical signs of disease and were exsanguinated 60 days following exposure. Their sera failed to exhibit rabies neutralizing antibody. Concurrently, 6 white Swiss mice were placed with, and received multiple bite exposures from these bats. The mice survived a 60-day observation period with no signs of clinical illness.

Inoculations of Other Animals and Fowl

Goats

Selected bat rabies strains were studied in 6 month old Spanish goats to determine this animal's susceptibility via the intracerebral route of inoculation (table 1).

One caprine (No. 1) inoculated with approximately 800,000 MIC LD_{50}'s of bat rabies strain 2084 died on the twelfth postinoculation day, exhibiting typical symptoms of rabies. Rabies virus was recovered from the brain of this animal.

Bat rabies strain 1248 was inoculated (approximately 630,000 MIC LD_{50}'s) into 2 goats (Nos. 2 and 3). Goat No. 2 exhibited symptoms of encephalitis on the ninth postinoculation day and died 2 days later. Goat No. 3 was lethargic and incoordinated, with a fixed stare on the tenth postinoculation day; 24 hours later a marked grinding of the teeth was noted, with progressive ascending type paralysis, and the animal died on the thirteenth postinoculation day. Mice inoculated with goat brain suspensions from these animals were positive for rabies, while the goats' salivary glands failed to reveal infection with the virus on replicate isolation attempts in mice.

Histopathological examination of brain tissue of three goats (Nos. 1, 2, and 3) revealed typical pathological findings, including meningo-encephalitis

neuronophagia perivascular infiltration glial nodules and Negri inclusion bodies

Strain 1063 was inoculated (approximately 500 000 MIC LD$_{50}$ s) into goat No 4 On the twelfth postinoculation day the animal refused water and food but after a 24 hr period it once again started to eat and drink and assume normal habits On the eighteenth postinoculation day the goat was challenged intracerebrally with approximately 8000 MIC LD$_{50}$ s of bat rabies virus strain 1063 The animal exhibited no central nervous system symptoms during a 50-day observation period and was sacrificed Serum specimens collected at time of inoculation and 41 and 50 days postinoculation were negative for neutralizing antibodies for rabies The mechanism of immunity observed in this animal manifested by its resistance to challenge in the absence of discernible neutralizing antibody cannot be explained

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brain passage)	I M	10^{6}	4/5	14 16 16 23 (61)	Negative	3/5	4/4
1009–13	I C	10^{4}	1/1	24	Positive	1/1	1/1
(Fifth mouse	S C	10^{6}	0/2	(33) (33)	Negative	0/2	1/2
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1248	I C	10	1/1	18	Negative	1/1	ND
(Third mouse	S C	10^{3}	2/2	20 21	Negative	2/2	2/2
brain passage)	I M	10	0/1	(35)	Negative	0/1	ND

Symbols ( ) all animals terminated after 30 days had remained asymptomatic for the observation period recorded ND test not performed

the brains of 2 of the rabbits exhibiting clinical rabies (1 I.C., 1 I.M.) nor from the brains of 6 asymptomatic rabbits surviving the observation period. Negri bodies were observed in Stovall Black stained sections of 2 of 19 rabbit brains examined (1 I.C., 1 I.M.). Rabies virus was recovered from the salivary glands (parotid and submaxillary pool) of only 1 (S.C.) of 8 rabbits from which tissues were harvested (1 I.C., 1 I.M., 6 S.C.).

Sera collected from 11 of the 13 symptomatic rabbits (2 sera not tested) exhibited positive serum neutralization indices. Sera of 3 of the 6 asymptomatic rabbits drawn the day of sacrifice were positive for rabies neutralizing substance. Two rabbits were negative and no serum specimen was collected from the sixth surviving animal.

Guinea Pigs

Nine guinea pigs (3 each I.C., I.P., and I.M.) received 8000 MIC LD_{50}s of bat rabies strains Nos. 768 and 1009-13. The 3 intracerebrally inoculated animals died of rabies with a mean incubation period of 15 days. Rabies virus was recovered from the brains of the 5 animals dying (3 I.C., 2 I.M.) and 2 of 4 subjected to histopathological examination of brain tissue revealed the presence of Negri bodies. All 3 of the intraperitoneally inoculated guinea pigs and 1 of the intramuscularly inoculated group survived a 61 day observation period with no clinical signs of disease.

Hamsters

Sixteen hamsters receiving 8000 MIC LD_{50}s of bat rabies virus strain No. 768 (5 I.P., 5 I.C., and 6 I.M.) died of rabies with a mean incubation period of 9 days. While rabies virus was recovered from the brains of all of these animals, Negri bodies were observed in only 2 (1 I.C., 1 I.M.) of 8 brains examined (4 I.C., 3 I.M., 1 I.P.).

Raccoon

One raccoon inoculated intramuscularly with approximately 630,000 MIC LD_{50}s of bat rabies strain 2084 remained asymptomatic for 62 days at which time it was exsanguinated. The animal's serum was negative for neutralizing antibodies to rabies and the brain yielded no viral agent when inoculated intracerebrally in mice.

One Day Old Chicks

Five of six 1 day old chicks inoculated intracerebrally with bat rabies strains 1009-13 and 1248 survived periods of from 46 to 107 days with no clinical evidence of disease. The sixth bird developed clinical symptoms of rabies including incoordination and leg weakness on the twenty ninth postinoculation day, followed by paralysis on the thirtieth day, at which time it was sacrificed (TABLE 3). Serum specimens drawn from all 6 inoculated birds on the day of sacrifice were positive for rabies neutralizing antibodies.

Five control chicks inoculated intracerebrally with normal mouse brain suspension were sacrificed after a 63-day observation period with no signs of dis-

TABLE 3
EXPERIMENTAL INFECTION OF 24 HOUR CHICKS WITH BAT RABIES VIRUS

Bat brain strain No.	IC dose mouse LD	Chick No.	Serum tested: Day*	Serum tested: Log NI†	Remark
1009-13 (Fifth mouse brain passage)	$10^{3.6}$	1	46 107	4 0 3 5	Asymptomatic
		2	46	4 0	Asymptomatic
		3	30	3 5	Clinical rabies Virus recovered
		4	54	4 0	Asymptomatic
1248 (Third mouse brain passage)	$10^{5.4}$	5	65	4 0	Asymptomatic
		6	65	2 0	Asympotmatic
Controls	NMB‡	7-11	63	0 0-0 5	Five normals

* Postinoculation day
† Log neutralization index
‡ NMB normal mouse brain

ease and sera collected at the time of sacrifice were negative for neutralizing antibodies for rabies

Bats (Antrozous pallidus pallidus)

After 8 days acclimatization in the laboratory each of 10 asymptomatic *A pallidus pallidus* was inoculated intramuscularly with approximately 13 000 MIC LD_{50}s of bat rabies strain 2084 Of the 10 inoculated 1 bat died the first and 2 on the third postinoculation day and were discarded as nonspecific deaths Six of the remaining 7 bats died between the fifth and ninth postinoculation days It is noteworthy that 1 inoculated bat survived 20 days before death intervened Individual attempts to recover the rabies virus from brain and salivary gland tissue of these bats by intracerebral inoculation of mice were futile in spite of the appearance of characteristic pathology other than Negri inclusion bodies Bacterial sterility controls were also negative

DISCUSSION

Possible theoretical objection to serial passage of the various bat rabies virus strains in mice on the grounds that such passage may tend to cause some fixation of the virus is recognized However subsequent experimental infections by the subcutaneous route and isolation of the virus from salivary glands argue against any theory of virus fixation

Other workers[11, 12] have demonstrated that rabid hemophagous and fructivorous bats are capable of transmitting rabies and may recover and transmit the disease as asymptomatic carriers However we have been unsuccessful in

the brains of 2 of the rabbits exhibiting clinical rabies (1 I C, 1 I M) nor from the brains of 6 asymptomatic rabbits surviving the observation period Negri bodies were observed in Stovall Black stained sections of 2 of 19 rabbit brains examined (1 I C 1 I M) Rabies virus was recovered from the salivary glands (parotid and submaxillary pool) of only 1 (S C) of 8 rabbits from which tissues were harvested (1 I C 1 I M 6 S C)

Sera collected from 11 of the 13 symptomatic rabbits (2 sera not tested) exhibited positive serum neutralization indices Sera of 3 of the 6 asymptomatic rabbits drawn the day of sacrifice were positive for rabies neutralizing substance Two rabbits were negative and no serum specimen was collected from the sixth surviving animal

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Sixteen hamsters receiving 8000 MIC LD_{50}s of bat rabies virus strain No 768 (5 I P 5 I C and 6 I M) died of rabies with a mean incubation period of 9 days While rabies virus was recovered from the brains of all of these animals Negri bodies were observed in only 2 (1 I C 1 I M) of 8 brains examined (4 I C 3 I M 1 I P)

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While interspecific comparisons are not entirely valid the results obtained in other animals inoculated with the various bat rabies isolates used in the present study do not suggest an attenuation of the virus Apart from a reduced capacity for negrigenesis there appears to be little difference in our hands between the biological characteristics of bat virus strains studied and those characteristics normally associated with street virus Despite a tendency toward a negative Negri pattern there is a high degree of infectivity for several laboratory animals and for goats The fact that 4 of 8 rabbits could be readily infected subcutaneously with a mean incubated period of 22 5 days is of considerable interest In our hands the incubation period of rabies caused by street virus is from 15 to 30 days for rabbits when the virus is introduced into the brain Establishment of infection via the relatively less susceptible subcutaneous route of entry by the bat rabies virus attests to its invasiveness and virulence Also the numerous isolations of the virus from salivary glands of infected animals indicate that it is not a fixed virus

The failure of infected bats to transmit rabies to confined monkeys and other laboratory animals suggests that a certain inherent mechanical deficit existed that prevented these animals from transmitting the disease The thickness of the cartilaginous tissues of the ear and the skin layers of the guinea pig appeared to govern the extent of the penetrating wounds that *T mexicana* and *A pallidus* were capable of inflicting In no instance did *A pallidus* penetrate the skin of guinea pigs in bite experiments to sufficient depth to produce hemorrhage Only localized edema without extravasation of tissue fluids was noted This inability to produce a deep penetrating wound may be one of the reasons for our negative transmission studies

Previously reported[8] and contrary to the negative host transmission results however is the presence of antirabies neutralizing antibody in large numbers of and the isolation of rabies virus from asymptomatic bats collected in nature These data suggested that a certain tolerance or diminished virulence in the host virus relationship did exist and suggested further the possibility of infection and recovery among the indigenous bat population Asymptomatic carriers if capable of transmitting the disease would be a formidable problem from the standpoint of preventive medicine because of the lack of self limiting factors

Statistical analysis of our unpublished bat serologic data compiled during the last 4 years of study of multiple bat sera pools revealed that the probable estimate of incidence of rabies-neutralizing substances for bat populations of 2 species of Molossidae was 15 4 per cent with 95 per cent confidence limits of 12 5 per cent and 17 9 per cent and for 5 species of Vespertilionidae was 5 7 per cent with 95 per cent confidence limits of 2 0 per cent and 9 4 per cent Computations were based on normal approximations to the binomial distribution of percentages It is believed that some importance may be attached to the significantly lessened incidence of rabies neutralizing substances in the Vespertilionidae as compared with the Molossidae The migratory habits known for *T mexicana* whose population makes up a large percentage of the Molossidae (composed of 2 genera and 6 species) in contrast with the hibernating behavior exhibited by Vespertilionidae offer an essential physical require

our attempts to prove naturally infected and laboratory infected indigenous insectivorous bats (*T mexicana* and *A pallidus pallidus*) capable of transmitting the disease by natural means to monkeys under conditions of induced association or by biting experiments on guinea pigs and mice The bite of the rabid *T mexicana* was quite capable of penetrating the skin or ears of the contact animals Recovery of rabies virus from the brain and salivary gland tissues of the infected bats indicated their potential capabilities for transmission

In the induced association experiment involving infected bats and normal monkeys, the natural curiosity of monkeys caused them to examine a number of the infected bats Consequently the monkeys were bitten several times on their hands and fingers before they could relieve themselves of the bats The reaction on the part of confined monkeys after being bitten was to attack the bats tear them apart and at times consume them thereby affording opportunity for further exposure This type of experience is probably duplicated to a certain extent in nature by predatory animals in their search for food Indeed data gathered by Twente[13] suggests that predators are important limiting factors in the maintenance of *Tadarida* populations This suggested the possibility that insectivorous bats infected with rabies might be a reservoir for the maintenance of the sylvatic disease Such a hypothesis could explain some of the bizarre geographical and cyclic disease patterns in wildlife rabies where outbreaks of the disease occur in rapid succession in widely separated regions of the United States

Results obtained by banding[13] suggest that the pattern of movement behavior for insectivorous bats is an erratic one in that bats banded in one cavern and released elsewhere will infrequently return to their original habitat but will quite often fly to other caverns not in the direct line of flight from the point of original release These transient individuals if infected with rabies would appear to offer excellent exposure possibilities for predatory animals that inhabit the same caverns

However if a parallel may be drawn between the habits of predatory animals in nature and the action of confined monkeys of the laboratory in attacking and consuming infected bats ingestion does not appear to contribute to the mode of transmission and perpetuation of the virus in nature

We experienced no difficulty in infecting bats either intracerebrally or peripherally This lack of tolerance on the part of the indigenous bats for the bat rabies virus by peripheral inoculation does not suggest that the virus has existed among the Molossidae for a sufficient time to produce an established relationship as manifested by diminished virulence for the host However we were unable to recover rabies virus from a group of intramuscularly inoculated *A pallidus pallidus* that received viable virus based on simultaneous titration of the inoculum in mice that yielded an LD_{50} titer of $10^{-5.3}$ The absence of the virus in the dead animals may have been due to enzymatic destruction of the virus in the dead cells or to a phenomenon referred to as an autosterilizable neuroinfection a condition postulated in rabies among other neurotropic virus diseases by Levaditi Sanchis Bayarri and Schoen[14] which presupposes that the reaction of the host is so efficient that the virus and the host are destroyed No rational answer appears to be available immediately

Guinea pigs and white mice bitten numerous times by bats (*A pallidus pallidus*) experimentally infected with bat rabies virus failed to contract rabies although isolation of rabies virus from the salivary glands and mouth swabbings of a significant number of the rabid bats was accomplished The guinea pigs failed to develop demonstrable neutralizing antibodies to rabies virus

Experimental infections of monkeys goats rabbits guinea pigs hamsters mice and chicks with bat rabies virus were accomplished

Laboratory infection of 4 of 8 rabbits and numerous bats (*T mexicana*) by subcutaneous inoculation and isolation of the bat rabies virus from salivary glands of a number of the rabid animals indicate that this studied agent has not assumed the characteristics normally associated with those of fixation Neither is the virus of indigenous bat rabies considered to have assumed an abberrant form in this unusual host

Among bats (*T mexicana* and *A pallidus pallidus*) goats rabbits guinea pigs hamsters and mice experimentally inoculated with bat rabies virus none showed apparent recovery from clinical rabies

Regardless of our negative results in transmission experiments the repeated isolation of bat rabies virus from salivary glands and from mouth swabbings of infected bats emphasizes our opinion that persons bitten by bats should consider the incident as a potential rabies exposure and that these cases should be evaluated for vaccine prophylaxis

Acknowledgment

We express our indebtedness to Lt Col John M Lukeman MC Commanding Officer of the Fourth United States Army Medical Laboratory for his encouragement and cooperation which made it possible to carry out the work described in this paper

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ment for interspecies transmission with the known rabies infected vampire bats (*Desmodus sp*) of Mexico The social behavior of clustering according to species is quite selective for colonial bats, in this respect one might speculate that migrant infected members of the Molossidae, in competing with the indigenous Vespertilionidae in habitat selection, present the necessary opportunity for the chance infection of the latter

Although positive serologic findings previously reported have suggested that nonfatal infections might occur they are by no means proof that they actually do occur Certainly the lack of survivors among the laboratory infected bats does not support such a thesis

Experiments conducted by Pawan[11, 1] with both laboratory infected and naturally infected bats demonstrated the capabilities of the vampires and fruit eating bats to recover from a clinical infection and continue to be capable of transmitting the virus for prolonged periods

Among the scores of insectivorous bats that were inoculated by peripheral and intracerebral routes with varying doses of bat rabies virus we have yet to find a single individual exhibiting a frank illness followed by recovery based upon a 70 day observation period

The possibility of latency of infection should not be dismissed even though we were unable to observe this phenomenon under the conditions described in our experimental methods To public health veterinarians charged with the responsibility of control of rabies reservoir type animals latency of infection would be of great significance If indigenous insectivorous bats were true carriers of rabies virus there would be no self limiting mechanism to assist these disease control specialists

The irregular appearance and low incidence of Negri bodies in infections with the bat rabies strains studied emphasize the danger of relying upon microscopic examination alone in diagnosing bat rabies

Bat rabies awaits further extensive study and investigation for clarification Meanwhile in spite of our negative results in transmission experiments repeated isolation of bat rabies virus from salivary glands and from mouth swabbings of infected bats emphasizes our opinion that persons bitten by bats should consider such incidents as potential rabies exposures and these cases should be evaluated for vaccine prophylaxis

SUMMARY

Six strains of bat rabies virus isolated from naturally infected bats (*T mexicana*) were used in transmission experiments and in studies of the susceptibility of other animals to the virus

Monkeys in close confinement with groups of bats (*T mexicana*) experimentally infected with bat rabies virus and with a naturally infected group of bats did not contract rabies nor develop demonstrable neutralizing antibodies against rabies virus We observed biting of the monkeys by the rabid bats and ingestion of some of the bats by the monkeys and rabies virus was isolated from the salivary glands of a significant number of the rabid bats Susceptibility of the monkeys to fatal infection with bat rabies virus was later proved by intracerebral challenge

NEWER TOOLS FOR THE PREVENTION OF RABIES IN DOMESTIC ANIMALS

By Merlin L. Kaeberle
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The occurrence of rabies in domestic animals is a problem of major importance in the United States and in many other countries of the world. Of primary concern to the veterinarian and physician is the resulting exposure of many human beings to the dread disease. A large percentage of our human exposures is due to contact with rabid animals classified as domestic. The number of reported cases of rabies in the United States during the ten year period 1947 to 1956 is shown in TABLE 1, which also serves to indicate the high percentage of cases reported in domestic animals as compared to other species. The problem is also an economic one, since the number of cattle and other valuable animals that succumb to the disease is substantial. The loss of valuable breeding stock is a serious matter, for all too often these animals cannot be replaced even though an indemnification is paid. Consequently, prevention of the disease in these animals is of more than minor interest.

The development of prophylactic agents has been essential, for once clinical signs of illness appear, the animal invariably dies. For this reason, ever since inception of the modern era of rabies research scientists have looked to agents that would prevent the disease. Previously, particular emphasis was placed upon prophylactic treatment of animals and humans following exposure to the disease rather than upon pre-exposure immunization.

Extensive experimentation by Louis Pasteur and his associates in the early 1880s culminated in the immunization of dogs against the disease by 1884. This research, employing the use of vaccine in animals, led to the successful administration of vaccine to human beings in 1885 and 1886.[25, 39, 6] The basis for the Pasteur vaccine was a virus modified by serial passage through rabbits until it had become "fixed." The virus was further reduced in virulence by desiccation of the virus-infected rabbit spinal cords over caustic potash. During this same period Galtier[11, 12] was concerning himself with the protection of herbivorous animals against rabies. He subsequently administered street virus intravenously to sheep, challenged them with the homologous strain introduced subcutaneously, and reported an immune response. In 1888 Roux and Nocard[41] reported experimental studies in which rabbit brain emulsion containing live rabies virus was administered intravenously to cattle and sheep that were later challenged with street virus by the intra-ocular route. Live virus was also administered by Moncet[37] and by Krasmitski,[23] who concluded that it was a safe and efficacious method. Living fixed virus was administered to dogs by Schnurer,[43] who reported favorable results.

Modifications of the Pasteur vaccine were soon forthcoming as additional workers entered this field of research. Thus in the early 1890s Hogyes, working at the Pasteur Institute of Budapest, Hungary, developed a vaccine that bears his name. This vaccine, prepared from fixed virus and further

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TABLE 2

SUMMARY COMPARATIVE CANINE RABIES VACCINE EXPERIMENTS[79]

Vaccine	Route of inoculation	Rabies mortality	Per cent mortality	Period between vaccination and challenge	Author
Brain tissue (phenolized) Controls	S C —	6/52 41/57	11.5 78 [illegible]	12 months	Johnson[8]
Modified live virus (chick embryo) Brain tissue (phenolized) Controls	I M S C —	0/25 3/22 18/25	0 13.6 72	12 months	Koprowski and Black[30]
Modified live virus (chick embryo) Brain tissue (phenolized) Controls	I M S C —	3/25 8/19 21/23	12.0 42.1 91.4	24 months	Koprowski and Black[30]
Modified live virus (chick embryo) Brain tissue (phenolized) Brain tissue (ultraviolet irradiated) Brain tissue (benzene ether treated) Controls	I M I M I M I M —	0/37 0/30 0/31 0/30 18/33	0 0 0 0 54.6	24 months	Tierkel Kissling Eidson and Habel
Modified live virus (chick embryo) Brain tissue (phenolized) Brain tissue (ultraviolet irradiated) Controls	I M I M I M —	0/30 8/34 7/30 31/36	0 23.6 23.3 86.1	3[illegible] months	Tierkel Kissling Eidson and Habel

Symbols S C subcutaneous I M intramuscular

activated virus vaccines have been proposed and developed their inadequacy has been illustrated by the never ending attempts to develop a better prophylactic agent The immunizing potential of some of these vaccines has been quite satisfactory and their value in the control of canine rabies has been proved However other vaccines have been found to be lacking in antigenic activity[34,66] There are still other disadvantages to the use of rabies vaccine of nervous-tissue origin A significant number of nervous type reactions result from the administration of vaccines of this type This is usually exhibited as a postvaccinal paralysis that may be mild and transient or so severe as to cause the death of the animal A second disadvantage is the rather limited duration of the immunity conferred by one injection of vaccine containing inactivated virus A one year period is most commonly accepted although immunity probably persists for a longer period in some animals and for a shorter time in others

The last decade has been a period of marked progress in the field of rabies immunology and this is due primarily to the development of a modified live virus vaccine (Flury strain) grown in chicken embryos Leach and Johnson[35] isolated this strain of virus from the tissues of a girl who had died of rabies Johnson subsequently carried the virus through 136 intracerebral passages

TABLE 1

REPORTED CASES OF RABIES IN THE UNITED STATES 1947 to 1956[50]

Year	Domestic animals	Other animals	Man
1947	8192	728	26
1948	7676	819	13
1949	6395	1192	10
1950	6526	1375	9
1951	6621	1387	14
1952	6758	1674	21
1953	7344	1479	14
1954	5577	1697	8
1955	3924	1915	5
1956	3757	2079	10

reduced in virulence by a multiple dilution method has been used extensively for immunizing domestic animals The results of these immunizations were summarized by Aujesky[1] who concluded that the vaccine was quite effective Experimental work conducted by Meissner[36] in which fixed virus was administered to calves and sheep by various routes indicates that immunity was induced by several doses of vaccine containing virus from freshly harvested brain tissue material

A group of vaccines that have been used extensively in the prophylactic antirabies treatment of man and animals are those containing inactivated virus Since there are many vaccines of this type only a few will be mentioned Phenolized vaccine first developed by Fermi[9, 10] as a prophylactic agent for use in man has received wide application in domestic animals The vaccine was modified by Semple[47] in 1911 and tested in animals prior to its extensive use in humans Umeno and Doi[49] adopted this type of vaccine for use in immunizing dogs by a single injection technique and the results achieved from administering the vaccine to about 15 000 dogs were quite promising In 1922 Eichorn and Lyon[6] introduced the method into the United States where they used it experimentally Based upon evidence presented by these investigators the vaccine was adopted for use by a number of health departments but it was necessary to alter the method of vaccine preparation slightly when Schoening[44] demonstrated the presence of live virus in it

As research progressed other agents were employed for the inactivation of rabies virus in the preparation of vaccines Kelser[3] prepared an antirabies vaccine by treating fixed virus with chloroform The efficacy of this vaccine was demonstrated by Kelser by Schoening[45] and by Leach[34] A departure from chemical inactivation was made by Hodes Lavin and Webster[18] in 1947 when they used ultraviolet irradiation to inactivate fixed virus Habel[17] also prepared a vaccine of this type and reported on its potency as an immunizing agent This vaccine was incorporated in duration of immunity studies conducted at the Communicable Disease Center Atlanta Ga and the results are indicated in TABLE 2 However the irradiated vaccines have never received extensive use in the field

Although many modifications of the original Pasteur vaccine and the in

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Vaccine	Route of inoculation	Rabies mortality	Per cent mortality	Period [illegible]	Author
Brain tissue (phenolized)	S C	6/52	11 5	12 months	Johnson[31]
Controls	—	41/52	78 9		
Modified live virus (chick embryo)	I M	0/25	0	12 months	Koprowski and Black[30]
Brain tissue (phenolized)	S C	3/22	13 6		
Controls	—	18/25	72		
Modified live virus (chick embryo)	I M	3/25	12 0	24 months	Koprowski and Black[30]
Brain tissue (phenolized)	S C	8/19	42 1		
Controls	—	21/23	91 4		
Modified live virus (chick embryo)	I M	0/32	0	24 months	Tierkel Kissling Eidson and Habel
Brain tissue (phenolized)	I M	0/30	0		
Brain tissue (ultraviolet irradiated)	I M	0/31	0		
Brain tissue (benzene ether treated)	I M	0/30	0		
Controls	—	18/33	54 6		
Modified live virus (chick embryo)	I M	0/30	0	39 months	Tierkel Kissling Eidson and Habel
Brain tissue (phenolized)	I M	8/34	23 6		
Brain tissue (ultraviolet irradiated)	I M	7/30	23 3		
Controls	—	31/36	86 1		

Symbols S C subcutaneous I M intramuscular

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in day old chicks. After two additional passages in chicks Koprowski and Cox were successful in adapting the virus to the developing chicken embryo.[39] The serial passage was then continued in embryonated eggs with a significant change in the virulence of the virus noted between the fortieth and fiftieth egg passage levels. At this stage the virus demonstrated a reduced virulence for hamsters and guinea pigs and a loss of virulence for rabbits and dogs when administered parenterally. Further experimental work was conducted to determine whether or not the virus had retained its antigenicity. A group of dogs inoculated with the virus material and later challenged with virus of the NYC strain demonstrated a good immunogenic response to the virus.[5] This finding was the basis for the production of vaccine prepared from the fortieth to fiftieth egg passage of the Flury strain virus. It is commonly known as low egg passage (LEP) modified live virus Flury strain and has been extensively tested in the laboratory and in the field.

The development of the Flury strain vaccine initiated extensive experimental activity. As with all new biological products the use of this vaccine both experimentally and in the field raised the usual questions concerning its *safety and efficacy.* *A number of experiments were designed to demonstrate* the relative effectiveness of the rabies vaccines commonly used in this country for protecting dogs against rabies. A summary of these studies is given in TABLE 2. In this table are included the results of the important challenge experiments conducted to evaluate rabies vaccines in dogs. A comparison of brain tissue vaccines with modified live virus vaccine Flury strain indicates that all vaccines tested induced some degree of resistance to challenge with street virus. However it appears that the modified live virus is a superior antigen. The 39 month duration of immunity study conducted by Tierkel *et al.*[48] at the Communicable Disease Center shows quite conclusively that modified live virus vaccine Flury strain produces a significantly greater degree of immunity than do inactivated virus brain tissue vaccines 39 months after vaccination.

Studies concerning the efficacy of modified live virus rabies vaccine were not confined to the laboratory. Limited field trials had been conducted prior to 1950 when the Expert Committee on Rabies of the World Health Organization (WHO) Geneva Switzerland recommended a demonstration program of rabies control.[7] This was to include the mass immunization of dogs with chicken embryo vaccine. The demonstration was carried out in the state of Israel where rabies had been a continuous problem for several years preceding 1950 despite control efforts. The mass immunization of dogs with modified live virus vaccine in conjunction with other control measures reduced the number of reported rabies cases to less than ten yearly during 1951 1952 and 1953.[22] The effectiveness of mass immunization of dogs with chicken embryo vaccine was again demonstrated in the Federation of Malaya. Although rabies had existed there since 1924 a federally sponsored rabies control program including mass canine immunization initiated in 1952 practically eliminated the disease from the country.

Modified live rabies virus has been in use in the state of New York since 1950. During that period more than 300 000 dogs have been administered

TABLE 3
EFFECTIVENESS OF RABIES VACCINES IN PUPPIES*

Vaccine	Results of challenge	
	Ratio [illegible]	Percent deaths
Modified live virus (LEP)	6/20	30
Brain tissue (phenolized)	6/20	30
Controls	11/11	100

* Puppies 2 to 4 months of age at time of vaccination The time between vaccination and challenge varied from 54 to 242 days

the vaccine at canine vaccination clinics The routine investigation of every reported case of canine rabies during that same period has revealed that only four of those dogs later succumbed to rabies despite the enzootic occurrence of the disease in the state

The immunogenic response of puppies to the antigenic stimulus of rabies vaccine has long been questioned The minimum age at which dogs might be successfully immunized against the disease was a matter of opinion Koprowski conducted studies utilizing two- and three month old puppies that were administered LEP Flury strain vaccine but the findings were rather inconclusive Kissling and his associates[6] carried out an experiment in which two- to four month old puppies from nonimmune dams were administered rabies vaccines and were later challenged The results of this study are shown in TABLE 3 and indicate that immunity was induced by the vaccines However the findings of the experiment hinted that puppies do not respond as well to an antigen as do adult dogs In 1956 a study was conducted by state and CDC veterinarians in the New York State Department of Health Albany N Y in cooperation with workers at Lederle Laboratories Division American Cyanamid Company Pearl River N Y Puppies ranging in age from 5 to 16 weeks were administered either LEP Flury or HEP Flury vaccine and five months later they were challenged with NYC strain virus The results of the challenge are given in TABLE 4 while there was protection against exposure the degree of immunity was not of a level that might be expected in adult animals In TABLE 5 the test animals are grouped by age at the time of vaccination and the results of challenge are shown It is thus quite well demonstrated that puppies less than 11 weeks of age do not respond as well to vaccine

TABLE 4
RESPONSE OF YOUNG DOGS TO CHALLENGE

Vaccine	Results of challenge		
	Number injected	Number dying	Percent
Modified live virus (LEP)	27	14	51 9
Modified live virus (HEP)	23	10	43 4
Controls	28	26	92 8

TABLE 5
EFFECTIVENESS OF RABIES VACCINE IN PUPPIES AT VARIOUS AGE LEVELS

Age at vaccination	Rabies mortality ratio	Percent mortality
5 to 7 weeks	11/18	61.0
8 to 10 weeks	10/16	62.5
11 to 16 weeks	3/16	18.7
Control 5 to 16 weeks	26/28	93.0

as do older animals. In young animals there appears to be a maturation factor that must be present to permit the type of antigenic response found in older animals.

The occurrence of rabies in cats and the immunization of this species against the disease also has been a matter of study during the last decade. Information concerning the epidemiology of rabies in cats and the effectiveness of rabies vaccines in protecting these animals against the disease has accumulated gradually. Cats are probably unimportant vectors of the disease in nature due to a number of factors. Recent studies[5] have shown that cats and kittens are more resistant than dogs and puppies to challenge with street virus by the peripheral route. Also, the inherent nature of cats and their behavior probably serves to limit the possibility of their exposure. While this is the case, it appears that the great majority of cats that develop rabies exhibit the furious form of the disease. The individual rabid cat therefore is a threat to man and other animals.

Laboratory studies concerned with the response of cats to rabies vaccines have demonstrated the effectiveness of these prophylactic agents. Koprowski[7] vaccinated cats with LEP Flury vaccine and subjected these animals to challenge 39 days later. The results of that study show that the vaccine induced a significant degree of protection. In an experiment conducted in the state of New York[5] it was shown that LEP Flury vaccine, HEP Flury vaccine and phenolized brain tissue vaccine provide an antigenic stimulus to impart a significant degree of immunity 70 days after vaccination (TABLE 6). However, the results would indicate that the nervous tissue vaccine is probably

TABLE 6
EFFECTIVENESS OF ANTIRABIES VACCINES IN PROTECTING CATS AGAINST RABIES

Vaccine			Response to challenge		
Type	Dose	Route	Number injected	Survived: Number	Survived: Percent
Controls	—		26	4	15.4
Modified live virus (LEP)	1.5 ml	I.M.	26	18	69.2
Modified live virus (HEP)	1.5 ml	I.M.	24	18	75.0
Brain tissue (phenolized)	3.0 ml	S.C.	27	26	96.3

Symbol: I.M., intramuscular; S.C., subcutaneous.

a superior antigen in adult cats Experiments conducted thus far have not provided data concerning the duration of immunity in cats

The safety of rabies vaccines when administered to cats has also received consideration Although the safety of brain tissue vaccines for felines has been little studied phenol does not appear to be a serious problem[3,4] Accumulated experimental data indicate that cats under six months of age should not receive modified live virus rabies vaccine Young animals are apparently more susceptible and an occasional kitten will succumb to LEP virus inoculated peripherally Although this age factor exists the maturation point after which greater resistance is demonstrated has not been determined in cats

The occurrence of rabies in cattle and sheep has been a problem for many years with the most emphasis placed upon prophylactic treatment of exposed animals The epizootiology of rabies in cattle was little considered prior to outbreaks of vampire bat rabies in the cattle of Central and South America Added impetus to these studies was given by the occurrence of bovine rabies in the United States caused by a spillover from the wildlife reservoir Cattle are apparently quite susceptible to rabies virus This is confirmed by observations of the high mortality following field exposures and by the susceptibility to LEP Flury virus[5,31] and experimental challenge inoculations[13,31] These findings have increased our knowledge of the disease in this species and brought further progress in rabies prophylaxis

The greater part of the recent experimental work concerned with the immunization of cattle against rabies has been carried on in connection with the modified live virus vaccines Since LEP Flury vaccine had proved to be so effective in the immunization of dogs there existed the possibility of its use in cattle Gomez Black and Koprowski[13] instituted a study in Colombia to determine the efficacy of LEP and chloroform inactivated rabies vaccine of nervous tissue origin (Kelser method) Extensive serum neutralization tests and challenge procedures were incorporated in this study which demonstrated the ability of both vaccines to induce immunity in cattle Shortly thereafter Schroeder and his colleagues[46] conducted an extensive field study in Honduras Costa Rica and Guatemala with particular emphasis on the immunization of cattle against vampire bat rabies In these studies the immunogenic potency of LEP Flury vaccine was shown quite conclusively However field use of the vaccine demonstrated a susceptibility of cattle to this virus for it was deemed to be the cause of paralysis and even of death in an occasional animal Since its safety was questionable the use of the vaccine in the bovine was contraindicated and its use largely discontinued

The search for a prophylactic agent that could be used successfully in cattle continued HEP Flury strain vaccine was the next to be investigated Continued serial passage of the Flury strain virus in embryonated chicken eggs had resulted in a further change in the virulence of the virus at egg passages 176 and 187 While suckling mice would still succumb to the virus when it was administered intracerebrally it no longer would kill adult mice The virus was also found to be apathogenic for rabbits and dogs by the same route Since there was a possibility of using this for the immunization of cattle Koprowski Black and Johnson[31] conducted laboratory studies to ascertain the

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in use for several years Improvements in this method of assay were made to give more consistent results from the testing of inactivated virus vaccine Among the accomplishments has been the adoption of a standard challenge virus for use in testing vaccine potency Since the potency mouse test could not be applied to the modified live virus vaccines it was necessary to develop a potency guinea pig test to be used for this purpose This was accomplished and has now been a standard method for several years These tests have served to provide better vaccines for the immunization of domestic animals

The past decade has thus been a period of great progress in the field of rabies prophylaxis in domestic animals We now have tools that if properly used will serve to reduce greatly the incidence of the disease in this group of animals

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immunogenic potency of this virus in cattle. This together with field studies[4] served to evaluate the vaccine in this species. The value of this antigen for immunizing cattle has thus been shown and the vaccine is now used quite commonly for this purpose in many areas of the world.

The postexposure treatment of domestic animals is a practice that has been carried on for many years. In fact, the earlier research was concerned primarily with the use of prophylactic agents following exposure. This continued to be the case until the advent of mass canine vaccination. Vaccines have been used widely in a number of European countries as post exposure treatment. Although a substantial amount of information has accumulated from the administration of many types of vaccines, it is difficult to draw conclusions due to the nature of the disease and the type of data available.

The possibility of inducing passive immunity by injections of antirabic serum was proposed by Babes and Lepp in 1889. The use of rabies antiserum was not commonly practiced during the ensuing years since its efficacy was questioned. However, Habel[14] reports that during this period a number of workers including Marie, Fermi, Proca, and Shortt were investigating its value as a prophylactic agent. The studies of Hoyt and Gorley[19, 20] and of Habel[15] in which seroprophylaxis was used to immunize mice served to revitalize efforts concerned with this approach. Additional experiments with guinea pigs demonstrated the value of antiserum in protecting laboratory animals. Koprowski has conducted extensive studies with hyperimmune serum in laboratory animals and dogs in the development of a product for human use. Although the preventive effect of antirabies serum has been shown, it is not generally used for treatment of animals. This is due to its cost and to the conditions under which many exposures of domestic animals occur.

The proper handling of dogs and cats exposed to the disease by a known rabid animal has long been a problem. It has been a usual practice to destroy such animals rather than to risk their acquiring the clinical disease and exposing other animals and possibly human beings as well. The alternative was to hold the animal in strict isolation for a period of 6 months. Recognition has now been given to postexposure treatment by using antirabies serum followed by 1 to 3 doses of chicken embryo vaccine or 14 injections of nervous tissue vaccine.[8] If the animal has been vaccinated within 1 year with nervous tissue or Kelev strain vaccine or within 3 years with LEP Flury vaccine, it is acceptable procedure to revaccinate the animal and place it in quarantine for 30 days. This serves to indicate the faith placed in rabies vaccines.

An inactivated rabies vaccine of duck embryo origin was recently released for use in humans. Clinical and laboratory results reported by Peck, Powell and Culbertson[40] indicate that the antigenic stimulus provided by this vaccine is quite satisfactory. It would seem that a vaccine of this type may have indications for use in domestic animals. Additional experimental work will be required to determine the efficacy of the vaccine in these species.

The development and use of rabies vaccines have been discussed, but marked progress has also been made in the potency testing of vaccines. In the past decade Habel and Wright[16] modified the potency mouse test that had been

in use for several years Improvements in this method of assay were made to give more consistent results from the testing of inactivated virus vaccine Among the accomplishments has been the adoption of a standard challenge virus for use in testing vaccine potency Since the potency mouse test could not be applied to the modified live virus vaccines it was necessary to develop a potency guinea pig test to be used for this purpose This was accomplished and has now been a standard method for several years These tests have served to provide better vaccines for the immunization of domestic animals

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Discussion of the Paper

Ernest S Tierkel (*Communicable Disease Center Public Health Service Atlanta Ga*) It seems entirely appropriate at this point to illustrate by means of field experience in rabies control the effects of this research in animal vaccination Two examples of the many successful immunization programs of the last ten years may be cited here Rabies in Memphis Tenn had been at a substantially high simmering level for years when it exploded into epidemic proportions in the early spring of 1948 despite an excellent program for the collection of stray dogs The organization of an intensified mass canine immunization campaign began swiftly In a single week in mid April 23 000 dogs were vaccinated in 70 emergency inoculation clinics strategically located throughout the city and county In the ensuing months positive reports began to drop until the last case of animal rabies and the last human antirabies treatment were reported in July (TABLE 1) The elimination of rabies from both city and county was a new and refreshing experience for Memphis

A similar campaign was conducted in Houston Texas in the fall of 1954 From 1945 to 1954 Houston had been plagued by an annual average of 300 animal cases with 14 human deaths During 1953 and 1954 the animal cases rose to an average of 40 per month In the last days of September 1954 an intensified 4-day program was carried out in which 45 000 dogs were vaccinated in 91 emergency clinics throughout the city and county Here again the success

Table 1
Animal Rabies Cases in Memphis Tenn 1948

January	13
February	24
March	41
April	28—Mass immunization campaign
May	5
June	2
July	2
August	0
September	0
October	0
November	0
December	0

TABLE 2
ANIMAL RABIES CASES IN HOUSTON TEXAS

1954	September	34—Mass immunization campaign
	October	33
	November	25
	December	17
1955	January	21
	February	15
	March	18
	April	13
	May	9
	June	14
	July	2
	August	3
	September	1
	October	0

of this program can be seen by the gradual decrease in cases from October 1954 to October 1955 (TABLE 2)

Progress achieved in the over all national incidence of rabies during the past decade is reflected in the annual reported cases, as shown in TABLE 3 The total number of cases in 1946 was 10 872 This declined steadily to 5 844 cases for 1955 a decrease of one half By the same token the human cases dropped from 22 in 1946 to 5 in 1955 a decrease of 77 per cent Although in most parts of the country the dog still accounts for most of the cases and remains the most important vector of the disease the striking decline in canine cases from 8384 in 1946 to 2657 in 1955 (68 per cent) is eloquent testimony to our efforts to control the disease during the past 10 years

We now come to measures taken for the prevention of rabies in man Over the years one of the problems in the specific biological therapy of exposed persons was the ineffectiveness of vaccine in cases of severe bite especially those involving the region of the head when the incubation period was too short to allow for the development of immunospecific protection We have now largely solved this problem by the production of hyperimmune rabies antiserum which can confer sufficient passive immunity during that critical early stage before the vaccine can stimulate active protection Convincing experimental evidence from challenge tested laboratory animals (Habel 1945 Koprowski *et al* 1950) and from antibody studies (Atanasiu *et al* 1956) and natural exposure studies in man (Baltazard and Bahmanyar 1955) have established the value of the antiserum when it is followed by the usual course

TABLE 3
REPORTED CASES OF RABIES IN THE UNITED STATES

Year	Dog	Wildlife	Cat	Livestock	Man	Total
1946	8 384	956	455	1 055	22	10 872
1955	2 657	1 915	343	924	5	5 844

The United States Department of Agriculture

of vaccine One serum injection of 0 5 ml /kg of body weight is given as soon as possible after the bite Since this is a horse erum product precautions should be taken to avoid anaphylactic reactions by testing the patient for sensitivity Antihistamines ACTH or cortisone seem to be useful when serum sickness occurs

The extent of the over all rabies problem in the United States can be measured by the 50 000 human exposures occurring annually each of which requires the expensive painfully long series of 14 to 21 daily vaccine treatments a therapeutic prophylactic regime requiring greater professional medical care than many other infectious diseases An important phase of this problem is the occasional occurrence in exposed persons of postvaccinal encephalitic neurologic, and paralytic complications following administration of the prescribed series of rabies vaccine treatments These reactions are believed to be tissue specific isoallergic phenomena Apparently they are related not to the rabies antigen present but rather to some encephalitogenic component of the nerve tissue in the vaccine They occur most frequently after the seventh inoculation and most often in persons who have had previous Pasteur treatment Reports on the frequency of these complications vary for example 1 in 527 (Cook *et al* 1955) 1 in 600 (Pait and Pearson 1949) 1 in 7025 (Appelbaum *et al* 1953) 1 in 7200 (Sellers 1948) 1 in 8500 (McKendrick 1940) Experiments are currently proceeding in order to find new approaches that will lead to an effective vaccination regimen without danger of damage to the central nervous system

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PROPHYLAXIS AGAINST RABIES IN HUMANS

By John P Fox

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Measures intended to prevent rabies in man may be divided into two basic categories those taken before and those taken after the infecting bite His torically since the ancient realization of the relation of dogs to human rabies the first category has embraced measures to avoid being bitten and since the early Nineteenth Century measures to control canine rabies In the second category local cleansing treatment of the bite wound has been prac ticed for centuries, acid treatment since 1830 [1] Specific active immunization following suspected exposure has been practiced since the time of Louis Pasteur With two important exceptions that will be indicated shortly the foregoing states in broad outline the situation today with respect to the orientation of efforts to prevent human rabies

Important refinements of course have been introduced in the years since Pasteur but without change in this basic orientation These include the development and widespread utilization of canine immunization as an aid to control improvements in methods of diagnosing animal infections as an aid to defining more certainly the individuals at risk and development of a re liable method[2] for measuring vaccine potency to help ensure effective immuni zation Despite these important technical advances completely safe and reliable protection of man against rabies has yet to be achieved

Three recent developments however afford the basis for renewed hope The first represents another example of technical advance and consists of the possible substitution of vaccine prepared in avian embryos—chicken[3, 5] or duck[6, 7]—for the conventional types of vaccine that are prepared from central nervous system (CNS) tissue of infected animals Such a vaccine being essentially free of CNS tissue should not induce the tissue specific sensitiza tion postulated as the cause of the demyelinating reactions that too often result from the Pasteur treatment The remaining two recent developments repre sent the application of new principles or orientation One the introduction of hyperimmune serum [8] belongs in the after the bite category and is intended to protect the recipient by passively conferred antibody against a disease with such a short incubation period that active response would be too long delayed The other which technically is still in the developmental stage falls chiefly in the before the bite category and consists of the demonstration that once in duced active immunity resulting from either conventional Pasteur treatment or a primary course of avian embryo vaccine can be recalled by a single booster inoculum [4, 5] With embryo vaccine this provides the basis for possible safe long term immunization initiated well before exposure in high risk population groups It also permits abbreviated (single inoculation) and presumably safe postexposure treatment of those who previously have had a course of Pasteur treatment

This presentation will endeavor to describe briefly and clearly the salient

features of human antirabies prophylaxis at present The major effort however will be reserved for the three important and most recent developments just mentioned Those readers desiring a more detailed and fully balanced review of the general problem are referred to an excellent and very recent paper by Karl Habel[9]

The Situation After the Bite

Presentation of the new information in proper developmental sequence makes it necessary to ignore the obvious logical order and to begin with the situation that confronts us after the bite has been inflicted

Determination of exposure Because of cost inconvenience and actual hazard related to unnecessary treatment an essential first step is to determine the likelihood of exposure The first question is whether the saliva of the animal was in contact with freshly abraded or lacerated skin To answer this one must determine whether a recent abrasion existed whether the wound was really a bite rather than a scratch with the paw and if so whether the skin was really broken Next one must determine whether the saliva actually was infectious If the animal is not available for observation (that is if it escaped or cannot be identified) the presumption of infectiousness must prevail and specific treatment must be instituted Otherwise except in instances of very severe exposure that may be associated with a short incubation period specific treatment may await the outcome of diagnostic procedures including observation of the animal

Since Negri bodies (the detection of which is the most rapid diagnostic procedure) become more numerous and more certainly detectable as disease progresses diagnosis ordinarily is hastened by delaying sacrifice of the animal until signs of rabies are well developed Mouse inoculation which requires an additional 6 to 14 days is indicated when contact has been well defined and Negri bodies are not found since of all infected animals the brains of about 1? per cent may be Negri negative[10] While demonstration of Negri bodies or rabies virus in the brain proves that the animal was infected it does not follow necessarily that the saliva at the time of the exposure contact was infectious Not only does rabies virus completely fail to reach the salivary glands in many instances (in 25 per cent in one series of 28 proved canine infections[1, 11]) but it is not present in any case for more than a few days before obvious signs of disease appear The available evidence is undesirably scanty in view of the crucial importance of this point but it suggests that appearance of virus in the saliva is always associated with early signs of illness (that is at least a slight rise in temperature) and is usually followed within no more than 5 days by clearly recognizable illness[1, 11] The practical implication of this is that *if the animal is still well 5 to 7 days after having bitten there was no exposure to rabies* even if disease becomes evident in the next day or two One important exception to the foregoing relates to bats which should be captured and sacrificed immediately for virologic tests since the outcome of infection is unpredictable in these species

Treatment of the bite wound The fundamental consideration here is that

rabies virus may remain localized at the site of introduction for as long as twenty four hours. Treatment is directed at destroying or eliminating the virus and to the extent that it is successful it may completely prevent infection or so reduce the size of the infecting dose that the incubation period is extended. This simple but very important procedure should be carried out in all instances of animal bite without regard to the probability of rabies exposure.

Modern studies agree that the classic method of acid cautery is quite effective, especially when carried out within the first few hours after the bite. However other and less painful and disfiguring methods also are nearly as effective under experimental conditions, these include irrigation and scrubbing with soap or detergent solutions.[12, 13] Recent observations by Perez Gallardo[13] confirm the earlier work of Habel[14] and indicate that local infiltration of antiserum is of real value.

It must not be forgotten that bites may be infected with agents other than rabies virus and that appropriate measures should be taken to combat these (that is antitetanus and antibacterial precautions).

Passive immunity. Active response to antirabies vaccine requires time in order than effective levels of antibody may be achieved (perhaps 20 days from initiation of treatment) and obviously cannot be expected to prevent a disease that occurs after unusually short periods of incubation as is commonly the case in severely exposed persons. The obvious remedy for this situation is to supply the needed antibody at once by passive means. Perhaps because of the inconclusiveness of early observations[15, 16] until recently there has been little sustained interest in this approach. Experimental work demonstrated the high degree of protection afforded animals by systemically and even locally administered antiserum.[13, 14] Somewhat later, the importance of early use of serum (within seventy two hours) was demonstrated and it was shown also that the maximum protection of animals requires antiserum supplemented by vaccine.[8, 17]

Since 1948 partially purified hyperimmune antirabies serum has been utilized on an experimental basis in man. In Georgia its use was restricted to persons suffering severe and certain exposure less than 72 hours before seeking treatment. By early 1953 68 persons none of whom developed rabies had received antiserum. In each case a course of antirabic vaccine also was begun after injecting the serum.[18] In 1954 the opportunity finally occurred to carry out a controlled antiserum trial that had been planned since 1950 by the World Health Organization (WHO) Expert Committee on Rabies. A rabid wolf in Iran attacked and bit 27 persons of whom 17 were severely exposed by bites on the head. These latter were divided into 3 groups for treatment the groups receiving respectively only vaccine or vaccine plus 1 dose of serum or vaccine plus 2 doses of serum (the second dose given after a 5-day interval). The rabies mortality ratios for these 3 groups were 3 5 1 6 and 0 6 respectively.[19] Sera were collected at frequent intervals from these persons[20] and on an experimental basis[5, 21, 22] from unexposed volunteers receiving vaccine alone or with serum and have been tested for neutralizing antibody. The resulting data indicate that even a single dose of serum coupled with a full

course of vaccine ordinarily results in the continuous presence of antibody during the 50- or 60 day period of observation after injection of the serum However especially with marginally effective vaccination active response may be partially or even completely suppressed because of the antiserum this suppressive influence being more pronounced with 2 doses of serum than with a single dose Hence while the mortality experience in Iran was better (though not significantly so) in the group given 2 doses of serum it would seem safer on theoretical grounds to give but a single dose of serum and to couple it with vaccine of well established antigenic effect

Antibody solution of equine origin is now generally available and should be used in all instances of severe exposure In instances where rabies infection of the biting animal is not clearly evident vaccine can be deferred pending the outcome of the usual observation procedures in all other cases serum should be followed promptly by the vaccine Use of serum in instances of less serious exposure is not mandatory and in my own opinion should be discouraged because of the relatively great frequency of serum sickness observed

Active immunization (Pasteur treatment) If circumstances make it necessary to assume that exposure has occurred the use of antirabic vaccine becomes mandatory Unique among vaccines in that it is used *after* exposure has occurred its rationale depends on the stimulation of active response in time to prevent the usually slowly incubating infection from involving vital areas of the central nervous system (CNS) Since in the individual instance the incubation period cannot be predicted reliably and may be short rapidity of response is greatly to be desired Presumably for this reason the course of immunization employed from the time of Pasteur to the present usually has been very intensive although on a largely empirical rather than solidly experimental basis Recent evidence to be discussed shortly suggests that fewer but better spaced inocula will stimulate a response with at least equal rapidity and magnitude

All antirabic vaccines consist of relatively crude suspensions of infected tissue Until recently all were prepared from the CNS tissue of animals (in the United States usually that of rabbits) For the most part the virus in such suspensions is inactivated by phenol (as in Semple type vaccine) or by ultraviolet light Harris type vaccine containing live fixed virus is produced by one biological firm in the United States and a similar live virus vaccine is distributed by the Georgia State Health Department Atlanta Ga Since live fixed virus apparently does not multiply in man these live virus and inactivated vaccines may be regarded as essentially similar They should be effective in proportion to their antigenic content and the immunizing efficiency of the methods by which they are utilized Also they presumably possess equivalent capacities to cause undesirable complications such as sensitivity to the general animal protein immediate allergic reactions (in persons already sensitive to the animal protein perhaps because of a previous course of treatment) or finally demyelinating reactions of the CNS (or isoallergic encephalitis) due to the development of organ specific sensitivity to the CNS This latter complication is the most serious and is the major reason for restricting the use of vaccine to cases in which the risk of not using the vaccine is carefully

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TABLE 1

TIME OF APPEARANCE OF ANTIBODIES FOLLOWING PRIMARY IMMUNIZATION WITH HARRIS, SEMPLE AND HEP FLURY VACCINES

Inoculation data				Number of persons inoculated	Number of persons with antibody* at indicated days after first inoculation						Per cent responding†
Type of vaccine	Total dose and route	Number of inoculations	Interval (days)		0	10	15	20	30	60	
Harris	7.0 ml S.C.	14	1	9	0	2	9	9	9	9	100
Semple	6.0 ml S.C.	12	1	10‡	0	2	8	9	9	9	90
		4	5	19	0	6	17	19	19	19	100
HEP Flury	12 gm I.M.	2, 3 or 6	1-7	14	0	7	8	8	9	12	93
	20 gm I.M.	5-10	1 or 2	10	0	7	7	7	8	9	100
	0.99 or 1.32 gm I.M.	3 or 4	5	18	0	5	9	10	12	13	78 (100)
	2.64 gm I.M. plus 0.64 gm I.D.	4	5	8	0	5	7	7	8	8	100
	0.12 to 0.36 gm I.D.	3	5	49	0	23	31	39	38	36	92 (94)
	0.16 to 0.64 gm I.D.	4	5	19	0	8	14	16	18	18	95

* Antibody as indicated by survival of 3 or more of 6 mice inoculated with mixture of 1:4 final dilution of serum and 100 LD_{50} of virus.

† Based on presence of significant neutralization in at least 1 serum specimen; apparent discrepancies between percentage shown and maximum number positive on any 1 day are due to individual variations in time of relatively transient low level responses. Figures in parentheses are based on retesting sera found negative in the conventional test by means of the long incubation test.

‡ Sera from these 10 persons were obtained through the courtesy of M. M. Kaplan, Veterinary Public Health Section, World Health Organization. These are the same individuals as group K in TABLE 3.

vaccine only in marginally effective amounts, it became particularly necessary to determine the most efficient method of rapid immunization.

Numerous small groups of volunteers were given different courses of vaccine including, for comparative purposes, Semple and Harris type vaccines. In TABLE 1 are presented data as to the time of appearance of neutralizing antibody, and the over-all proportion responding in some of the groups manifesting the best responses. Although only the 2 most rigorous courses of Flury vaccine (with a total dose of 20 gm. or with combined intramuscular and intradermal inoculations) elicited a readily detected response in 100 per cent of vaccinees, it is interesting that, as measured by rapidity of response (the proportion with antibody by the tenth day), Flury vaccine did better (47 per cent of 118 persons) than Semple and Harris vaccines combined (26 per cent of 38 persons). *Also of note is the fact that 4 doses of Semple vaccine spaced at 5 day intervals* did at least as well as 12 or 14 daily doses of Semple or Harris vaccine. In TABLE 2 are shown the available data as to titers of neutralizing antibody at 15 and 30 days after initiation of vaccination in some of the same groups of volunteers. The best antibody titers among those given HEP Flury vaccine were in the groups receiving the 20 gm. total dose intramuscularly or a course

evaluated as being greater than the danger of using it. This latter risk is variously estimated[23, 24] (the maximum estimated frequency is 1 in 600) but is greater in persons who have had one or more previous courses of treatment. Case fatality may be as great as 25 per cent but survivors usually manifest few permanent sequelae.[25]

The occurrence of these complicating reactions plus the incomplete effectiveness of specific prophylaxis has resulted in recent times in a very vigorous reinvestigation of human immunization. Passive immunity described above appears to afford an answer to the problem posed by the disease that occurs after a short incubation period. Elimination of the CNS specific tissue antigen and reduction in the capacity of the vaccine to induce sensitivity to the animal protein would resolve the problem of complications due to the vaccine. One obvious answer would be to grow the viral antigen in tissue other than CNS and if possible in a tissue poor in sensitizing proteins. Avian embryos provide a suitable host for viral growth from which CNS tissue can be readily eliminated; furthermore embryonic proteins are very poorly antigenic themselves. However, before vaccines of such different types can be recommended for general use *it must be shown that they are no less effective as viral antigens than those currently in use.*

Use of these vaccines in man has been studied independently[3, 7] and also on a world wide collaborative basis[1, 27] under the guidance of the WHO Expert Committee on Rabies. This latter study in particular is very comprehensive in scope and has undertaken to make unbiased comparative tests not only of vaccines of different types but also of various inoculation schedules of such vaccines and of the combined use of antiserum plus vaccine as already described. Unfortunately little of the WHO work with avian embryo vaccines has been completed at this time.

The avian vaccine most extensively studied in man is that made from chick embryos infected with the high egg passage (HEP) Flury strain of virus.[26, 7] This vaccine is used as a partially clarified suspension (40 to 80 per cent) of infectious chick embryo (often with penicillin and streptomycin added) with infectivity preserved by lyophilization and is given preferably in doses of 0.1 to 0.2 ml. intradermally or 0.5 ml. intramuscularly. More than 550 primary immunizations have been given to volunteers with various dose and inoculation schedules and more than 220 recall doses have been given to persons previously immunized with Flury vaccine or by the conventional Pasteur treatment.[3, 5] This fairly extensive use provides the basis for considerable confidence as to the primary safety of HEP Flury vaccine. Local reactions especially after intradermal inocula have constituted the chief but still not very considerable postvaccination complaint. Early work[3] made two points clear: (1) although alive the HEP Flury virus (like fixed rabies virus) is not able to multiply in man since response is directly related to the amount of vaccine inoculated and (2) using closely spaced intramuscular inocula excessive amounts of vaccine (12 to 20 gm. total dose of chick embryo) are required to elicit a satisfactorily uniform response. Since immune response is dependent entirely on the viral antigen actually injected and since this apparently is present in the

β-propriolactone.[7] This vaccine as of the moment has an apparent advantage over the HEP Flury vaccine in two or three respects. First, while it has been assumed on theoretical grounds that the Flury vaccine is free of neuroparalytic factors, the duck embryo vaccine actually has been found to be free in adjuvant tests in guinea pigs.[79] Second, the data reported for several lots suggest that in potency tests it consistently surpasses by two or more times the standard vaccine of the National Institutes of Health, Public Health Service, Bethesda, Md., whereas for reasons not clear Flury vaccine will not pass this test. Third, because of the potency test data, the duck embryo vaccine has now been licensed for commercial production and is generally available. Rather surprisingly, however, the comparable available data as to antibody response to a course (14 daily doses) of duck embryo vaccine, as shown at the bottom of TABLE 2, suggest that the level of human response is little better than response to a 4-dose course of Flury vaccine and considerably lower than that to Semple or Harris vaccines. It should be mentioned also that insofar as it can be ascertained from published reports, the primary safety of HEP Flury vaccine for man has been tested far more extensively by actual use in man than that of duck embryo vaccine. Direct comparisons of HEP Flury and duck embryo vaccines are now in progress.

Work with vaccines of CNS origin inactivated with phenol, as carried out in the WHO sponsored study,[1, 79] is summarized in TABLE 3 with respect to time of appearance of antibody. The chief point of interest is that the 1 and 3 dose schedules were grossly less effective than those of 12 or 14 daily doses or even than that of 7 daily doses. Generally speaking, the results of the schedules with daily doses compared favorably with those of similar schedules with Harris vaccine as already presented. However, there seems an obvious disparity, perhaps related to differences in vaccine potency, between the results

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Group designation	Vaccine	Single dose (ml)	Number of doses	Interval (days)	Number of persons	Number of persons with antibody on day					O[illegible] p[illegible]†
						7	10	14	21	28	
F	20% rabbit brain	3.5	1	—	10	1	3	4	3	4	50
B	20% rabbit brain	0.5	7	daily	10	1	[illegible]	8	9	9	90
K‡	20% rabbit brain	0.5	12	daily	10	1	2	9	9	9	90
L	5% goat brain	2.0	14	daily	10	NT§	0	8	10	10	100
N	5% goat brain	2.0	3	5	10	NT	1	1	5	6	70
T	20% rabbit brain	0.5	14	daily	6	NT	1	2	6	6	100

* Adapted from papers by Atanasiu et al.[79]

† As in TABLE 1.

‡ Group K is the same group as that mentioned in TABLE 1 as receiving 12 doses of Semple vaccine; the results shown in TABLE 1 were obtained in tests using 100 LD_{50} of virus rather than 30 LD_{50} as employed by the WHO collaborators.

§ NT = not tested.

TABLE 2

OBSERVATIONS ON LEVEL OF NEUTRALIZING ANTIBODY FOLLOWING PRIMARY IMMUNIZATION WITH HARRIS, SEMPLE, AND HEP FLURY VACCINES AND FOR COMPARISON DUCK EMBRYO VACCINE†

Type of vaccine	Total dose administered	Number of inocula	Interval (days)	Total number of persons	Days after first stimulum	Number of persons with antibody titer in indicated range of:*					
						<4	4-7	8-31	32-127	128-511	512+
Harris	7.0 ml S.C.	14	daily	9	15			2	4	3	
				9	30				2	3	4
Semple	2.0 ml S.C.	4	5	19	15	2		1	7	7	2
				19	30			1	4	9	5
HEP Flury	20.0 gm I.M.	5-10	1-2	6	15	2	1		3		
				6	30			3	3		
	0.99 to 2.64 gm I.M.	3 or 4	5	16	15	6	6	4			
				16	30	2	8	4	1	1	
	2.64 gm I.M. + 0.32 gm I.D.	4	5	8	15	1	1	2	3	1	
				8	30			3	5		
	0.12 to 0.96 gm I.D.	3	5	45	15	19	16	8		2	
				45	30	11	14	13	5	2	
	0.16 to 0.64 gm I.D.	4	5	19	15	5	7	6	1		
				19	30	1	8	6	2	2	
Duck embryo†	7.0 ml S.C.	14	daily	21	15	1	7	13			
				17	30	1	2	9	5		

* As measured by testing serial twofold dilutions of sera in groups of 6 mice per dilution using 100 LD_{50} of challenge virus per intracerebral mouse inoculum.

† The duck embryo data abstracted from the paper by Peck *et al.*[7] appear comparable in that the same methods of determining neutralizing antibody were employed.

of 4 intradermal doses alone or together with intramuscular doses with 5 day intervals. While the range of titers observed after the administration of Flury vaccine clearly overlaps those resulting from Harris or Semple vaccine the fact remains that no course of Flury vaccine so far employed has induced response fully equivalent to that following Harris or Semple vaccine with respect to both uniformity and antibody level. A further point to note is that the 4-dose course of Semple vaccine induced antibody titers in essentially the same range as did the 14-dose course of Harris vaccine. Unfortunately no comparable group was given a 14-dose course of the same lot of Semple vaccine.

The growth of fixed virus in duck embryos[*] provided the basis for the more recent type of avian embryo vaccine. Although first used as a live virus vaccine[6] the product as recently licensed is inactivated by treatment with

β propriolactone.[7] This vaccine as of the moment has an apparent advantage over the HEP Flury vaccine in two or three respects. First, while it has been assumed on theoretical grounds that the Flury vaccine is free of neuroparalytic factors, the duck embryo vaccine actually has been found to be free in adjuvant tests in guinea pigs.[29] Second, the data reported for several lots suggest that in potency tests it consistently surpasses by two or more times the standard vaccine of the National Institutes of Health, Public Health Service, Bethesda, Md., whereas for reasons not clear Flury vaccine will not pass this test. Third, because of the potency test data the duck-embryo vaccine has now been licensed for commercial production and is generally available. Rather surprisingly, however, the comparable available data as to antibody response to a course (14 daily doses) of duck embryo vaccine, as shown at the bottom of TABLE 2, suggest that the level of human response is little better than response to a 4-dose course of Flury vaccine and considerably lower than that to Semple or Harris vaccines. It should be mentioned also that, insofar as it can be ascertained from published reports, the primary safety of HEP Flury vaccine for man has been tested far more extensively by actual use in man than that of duck embryo vaccine. Direct comparisons of HEP Flury and duck embryo vaccines are now in progress.

Work with vaccines of CNS origin inactivated with phenol, as carried out in the WHO sponsored study[21, 22] is summarized in TABLE 3 with respect to time of appearance of antibody. The chief point of interest is that the 1 and 3 dose schedules were grossly less effective than those of 12 or 14 daily doses or even than that of 7 daily doses. Generally speaking, the results of the schedules with daily doses compared favorably with those of similar schedules with Harris vaccine as already presented. However, there seems an obvious disparity, perhaps related to differences in vaccine potency, between the results

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Group designation	Vaccine†	Size of dose (ml)	Number of doses	Interval (days)	Number of persons	Number of persons with antibody on day: 7	10	14	21	28	Overall percent responding†
E	20% rabbit brain	3.5	1	—	10	1	3	4	3	4	50
B	20% rabbit brain	0.5	7	daily	10	1	2	8	9	9	90
K‡	20% rabbit brain	0.5	12	daily	10	1	2	9	9	9	90
L	5% goat brain	2.0	14	daily	10	NT§	0	8	10	10	100
N	5% goat brain	2.0	3	5	10	NT	1	1	5	6	60
T	20% rabbit brain	0.5	14	daily	6	NT	1	2	6	6	100

Adapted from papers by Atanasiu *et al.*[22]
† As in TABLE 1.
‡ Group K is the same group as that mentioned in TABLE 1 as receiving 12 doses of Semple vaccine; the results shown in TABLE 1 were obtained in tests using 100 LD_{50} of virus rather than 30 LD_{50} as employed by the WHO collaborators.
§ NT = not tested.

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OBSERVATIONS ON LEVEL OF NEUTRALIZING ANTIBODY FOLLOWING PRIMARY IMMUNIZATION WITH HARRIS SEMPLE AND HEP FLURY VACCINES AND FOR COMPARISON DUCK EMBRYO VACCINE†

Type of vaccine	Total dose and route	Number of inocula	Interval (days)	Total number of persons	Days after first inoculum	Number of persons with antibody titer in indicated range of 1:x					
						<4	4–7	8–31	32–127	128–511	512+
Harris	7.0 ml S.C.	14	daily	9 9	15 30			2 	4 2	3 3	 4
Semple	2.0 ml S.C.	4	5	19 19	15 30	2 		1 1	7 4	7 9	2 5
HEP Flury	*20.0 gm* I.M.	*5–10*	*1–2*	*6* 6	*15* 30	*2* 	*1* 	 3	*3* 3		
	0.99 to 2.64 gm I.M.	3 or 4	5	16 16	15 30	6 2	6 8	4 4	 1	 1	
	2.64 gm I.M. + 0.32 gm I.D.	*4*	*5*	*8* 8	*15* 30	*1* 	*1* 	*2* 3	*3* 5	*1* 	
	0.12 to 0.96 gm I.D.	3	5	45 45	15 30	19 11	16 14	8 13	 5	2 2	
	0.16 to 0.64 gm I.D.	4	5	19 19	15 30	5 1	7 8	6 6	1 2	 2	
Duck embryo†	7.0 ml S.C.	14	daily	21 17	15 30	1 1	7 2	13 9	 5		

As measured by testing serial twofold dilutions of sera in groups of 6 mice per dilution using 100 LD_{50} of challenge virus per intracerebral mouse inoculum.

† The duck embryo data, abstracted from the paper by Peck *et al.*[7] appear comparable in that the same methods of determining neutralizing antibody were employed.

of 4 intradermal doses alone or together with intramuscular doses with 5 day intervals. While the range of titers observed after the administration of Flury vaccine clearly overlaps those resulting from Harris or Semple vaccine, the fact remains that no course of Flury vaccine so far employed has induced response *fully equivalent to that following Harris or Semple* vaccine with respect to both uniformity and antibody level. A further point to note is that the 4-dose course of Semple vaccine induced antibody titers in essentially the same range as did the 14 dose course of Harris vaccine. Unfortunately no comparable group was given a 14 dose course of the same lot of Semple vaccine.

The growth of fixed virus in duck embryos[5] provided the basis for the more recent type of avian embryo vaccine. Although first used as a live virus vaccine[6] the product as recently licensed is inactivated by treatment with

β propriolactone[7] This vaccine as of the moment has an apparent advantage over the HEP Flury vaccine in two or three respects First while it has been assumed on theoretical grounds that the Flury vaccine is free of neuroparalytic factors the duck embryo vaccine actually has been found to be free in adjuvant tests in guinea pigs[29] Second the data reported for several lots suggest that in potency tests it consistently surpasses by two or more times the standard vaccine of the National Institutes of Health Public Health Service Bethesda Md whereas for reasons not clear Flury vaccine will not pass this test Third because of the potency test data the duck embryo vaccine has now been licensed for commercial production and is generally available Rather surprisingly however the comparable available data as to antibody response to a course (14 daily doses) of duck embryo vaccine as shown at the bottom of TABLE 2 suggest that the level of human response is little better than response to a 4-dose course of Flury vaccine and considerably lower than that to Semple or Harris vaccines It should be mentioned also that insofar as it can be ascertained from published reports the primary safety of HEP Flury vaccine for man has been tested far more extensively by actual use in man than that of duck embryo vaccine Direct comparisons of HEP Flury and duck embryo vaccines are now in progress

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Group designation	Vaccine	Single dose (ml)	Number of doses	Interval (days)	Number of persons	Number of persons with antibody on day					Overall percent responding†
						7	10	14	21	28	
D	20% rabbit brain	3 5	1	—	10	1	3	4	3	4	50
B	20% rabbit brain	0 5	7	daily	10	1	2	8	9	9	90
K‡	20% rabbit brain	0 5	12	daily	10	1	2	9	9	9	90
L	5% goat brain	2 0	14	daily	10	NT§	0	8	10	10	100
N	5% goat brain	2 0	3	5	10	NT	1	1	5	6	70
T	20% rabbit brain	0 5	14	daily	6	NT	1	2	6	6	100

Adapted from papers by Atanasiu *et al* [1]
† As in TABLE 1
‡ Group K is the same group as that mentioned in TABLE 1 as receiving 12 doses of Semple vaccine the results shown in TABLE 1 were obtained in tests using 100 LD_{50} of virus rather than 30 LD_{50} as employed by the WHO collaborators
§ NT = not tested

with 3 doses in TABLE 3 and those with 4 doses of Semple vaccine shown in TABLE 1 in each case with a 5 day interval

To recapitulate briefly, when exposure must be presumed, active antirabic immunization is mandatory A major hazard is isoallergic encephalitis to avoid which embryo vaccines may provide the answer Duck embryo vaccine has been licensed whereas HEP Flury vaccine thus far has not been licensed Although promising results have been observed after modified dose schedules these must be confirmed before the usual 14 daily dose treatment is abandoned Management of exposure in persons who previously have had Pasteur treatment is a special situation now to be described

The Situation Before the Bite

Broadly speaking the preventive measures that should be taken before the bite fall into one or another of three main categories as follows those that help to minimize contact between potentially infected animals and man those directed at controlling rabies in domestic and wild animals and those that may afford man specific protection in advance of exposure For lack of space I shall pass over the first two categories *remarking only upon two re*latively recent developments The first of these is the availability and apparently superior usefulness of canine Flury vaccine[33] which because of the long duration of the resulting immunity is a potentially great adjunct to the control of canine rabies The second discussed at length elsewhere in these pages by K F Burns is the emergence of extensive rabies infection in herbivorous and insectivorous bats as a possibly important new factor in wildlife rabies

Measures falling into the third category the specific protection of man in advance of exposure have been possible in theory for a long time but have been rarely practiced because of the risk associated with receiving antirabic vaccine of the types heretofore available The advent of the presumably safe avian embryo vaccines makes possible a very great change in this situation and considerable work has been done already with the HEP Flury vaccine[4-6] as I shall try to indicate in the following summary The observations in question suggest that primary immunization with HEP Flury vaccine or by means of Pasteur treatment (presumably including that with duck embryo vaccine) provides a foundation immunity that conditions the recipient into responding rapidly to single stimuli given as much twenty or more years later Upon such a basis one can build a long term immunity by means of periodic booster inocula

About 64 persons who had received a primary course of HEP Flury vaccine were given single booster inocula of the same vaccine 5 to 48 months after the primary course the results are summarized in TABLE 4 It is of interest that small intradermal inocula elicited responses just as effectively as did the larger ones given intramuscularly Although definite failures of response (in 3 cases only) were observed only among those whose primary response had failed or was uncertain there was observed a definite correlation between the maximum antibody titer after the primary course and that after the booster This is shown in TABLE 5 and it suggests that in terms of antibody level at

TABLE 4

SUMMARY OF ANTIBODY RESPONSES OBSERVED IN PERSONS IMMUNIZED EXPERIMENTALLY WITH DIFFERING PRIMARY COURSES OF HEP FLURY VACCINE AND GIVEN SINGLE BOOSTER INOCULA OF THE SAME VACCINE AT FROM 5 TO 48 MONTHS AFTER PRIMARY IMMUNIZATION

HEP Fl y booste ot I m		R pon t pr ma y se	N mbe g boost	N mbe espo d g	Ra g f m m m r po se t t (1)†
D (g m)	R t				
0 001 0 16	I D	None	1	1	32
		Dubious	5	3	>32-96
		Yes	19	18(19)	<4 256
0 33	I M	Dubious	2	2	>4
		Yes	12	7(12)	>4-32
2 0	I M	None	4	2(3)	<4->256
		Dubious	11	11	<4-96
		Yes	10	10	>4->1024

Figures in parentheses include instances in which response was likely but because of inadequate serum specimens the conclusive quantitative test could not be done

† Results of <4 mean some mice were protected in the lowest serum dilution tested (1 4 fi al) but not enough to give a 1 4 titer use of the > sign means that because of inadequate erum pecimens retests to determ ne the definite end point could not be done

least best results with boosters are obtained after the more effective primary courses However without regard to the level of primary response nearly three fourths of the 42 persons responded to the booster with titers of 1 32 or better Some idea of the maintenance of antibody levels after primary and booster immunizations is given in TABLE 6 With respect to the primary course it seems clear that except for the less common individual with a primary response titer of 1 32 or better antibody persistence at a readily demonstrated level is likely to be brief While the same relation exists in the case of the postbooster persistence the pattern differs significantly in that the maximum titer after booster was usually 1 32 or better In practice therefore it would seem that adequate levels persist in most instances for at least two years after

TABLE 5

CORRELATION OF MAXIMUM ANTIBODY TITER AFTER HEP FLURY BOOSTER AND MAXIMUM TITER FOLLOWING PRIMARY COURSE OF VACCINE

M m m t body t t (1) ft primary se	T tal umb f pe so	N mbe f pe th m m m t body t t (1) ft boost ocul m				
		0	<4	4–7	8–31	32+
32+	5					5
8–31	5				1	4
4–7	6		1		1	4
<4	19	1	2	1		15
0	7	3	1		1	2
All	42	4	4	1	3	30

Symbol <4 as in TABLE 4

with 3 doses in TABLE 3 and those with 4 doses of Semple vaccine shown in TABLE 1 in each case with a 5 day interval

To recapitulate briefly, when exposure must be presumed, active antirabic immunization is mandatory A major hazard is isoallergic encephalitis to avoid which embryo vaccines may provide the answer Duck embryo vaccine has been licensed whereas HEP Flury vaccine thus far has not been licensed Although promising results have been observed after modified dose schedules these must be confirmed before the usual 14 daily dose treatment is abandoned Management of exposure in persons who previously have had Pasteur treatment is a special situation now to be described

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with respect to the management of treatment on the occasion of any subsequent new exposure to rabies. Deliberate use of Pasteur treatment as the foundation for maintenance immunity would not be recommended, obviously, because of the associated risk; therefore this aspect has not been explored in detail. However, when treatment has been required because of exposure, it is possible that profit may be taken from the misfortune and immunity maintained thereafter as suggested above. Indeed, proof that this may be done and guidance as to timing may be derived from the following data which bear more directly on managing the treatment of a new exposure.

A total of 136 persons with histories of previous Pasteur treatment, chiefly veterinarians but including some prisoner volunteers, were persuaded to submit to a single inoculation of HEP Flury vaccine, usually by the intradermal route, and to one preinoculation and several postinoculation bleedings. The results are summarized in TABLE 7. Definite response was demonstrated in 86 per cent of the total or, excluding 9 persons for whom the tests were not determinative (see footnotes to TABLE 7), in 92 per cent (117 of 127) of those properly tested. Furthermore, of the 10 definite failures, 6 were among 17 persons last treated 20 or more years previously. Although some variation

Table 8

Summary of Observations as to Persistence of Neutralizing Antibody After Pasteur Treatment

Number of treatments previous	Antibody level (1:)	Number of persons observed at indicated years after last treatment									
		<1	1–2	3–4	5–6	7–8	9–11	12–15	16–20	21–25	35–40
Only 1	32+	1		3		2	3	1	3		
	8–31	2	3	2	2			2	1	1	
	4–7						1				
	<4 or PLT		7	1	1	3	1	3	4	1	1
	0	2	2	3	5	1	4	5	7	6	3
	All pos	3	10	6	3	5	5	6	8	2	1
	All	5	12	9	8	6	9	11	15	8	4
Two or more	32+	1	6	4	4	6	1	1			
	8–31		1	1			1	1	2		
	4–7						2				
	<4 or PLT					2	2	1	2		
	0					1	1		2	1	
	All pos	1	7	5	4	8	4	5	4	0	
	All	1	7	5	4	9	5	5	6	1	
Only 1	Per cent positive	60	84	67	38	84	56	55	53	25	25
		73			57			54		25	
Two or more		100	100	100	100	89	80	100	67	0	
		100			89			78		—	

PLT means positive in qualitative long incubation test; in most such instances no quantitative tests were made. Also <4 as in TABLE 4.

TABLE 6

RELATION BETWEEN MAXIMUM ANTIBODY TITER AFTER PRIMARY COURSE AND AFTER BOOSTER OF HFP FLURY VACCINE AND PRESENCE OF ANTIBODY AT SUBSEQUENT INTERVALS

Titers following	Maximum titer (I)	Presence of antibody at indicated intervals: 5 Months, Total No.	Number with titer: <4	4 or >	12 Months, Total No.	Number with titer: <4	4 or >	24 or more months, Total No.	Number with titer: <4	4 or >
Primary course	32+	2	1	1	2		2	4		4
	8–31	3	2	1	4	1	3	1	1	
	4–7	7	7		2	1	1	3	3	
	<4	8	7	1	16	15	1	6	6	
Booster	32+				17	2	15	8	1	7
	8–31				1	1		0		
	4–7				4	4		2	1	1
	<4				1	1		1	1	

The symbol <4 in this case may include sera devoid of antibody since many were negative in 1:4 final dilution and not tested in lower dilution.

a booster. Second, third, and even fourth (in one instance only) boosters have been given but, thus far, to an insufficient number of persons to permit definition of the optimum spacing.

As stated previously, Pasteur treatment itself provides a foundation immunity upon which a program of maintenance immunity also may be built and which, in any event, carries important and long persisting implications.

TABLE 7

SUMMARY OF ANTIBODY RESPONSES TO HFP FLURY BOOSTER INOCULA* GIVEN TO PERSONS WITH HISTORIES OF PREVIOUS PASTEUR TREATMENT

Year last treatment	Number of persons	Persons responding†: Number	Percent	Range of maximum response titer ‡ (I)
unknown	5	4	80	>32–>1,024
35–40	4	1	25	128
20–26	13	9 (10)	69 (77)	<4–>4,100
15–19	21	20 (21)	95 (100)	<4–1,500
10–14	20	20	100	32–2,048
5–9	34	31 (33)	91 (97)	4–16,400
3–4	15	14 (15)	93 (100)	45–3,640
1–2	18	16 (17)	89 (94)	38–>4,096
<1	6	2 (5)	33 (83)	18–282
All	136	117 (126)	86 (93)	<4–16,400

* Inocula usually intradermal and in amounts ranging from 0.08 to 0.16 gm.; in 15 persons, however, intramuscular inocula of 2.0 gm. were given.

† Use of parentheses has same meaning as in TABLE 4.

‡ Use of < and > has same meaning as in TABLE 4.

with respect to the management of treatment on the occasion of any subsequent new exposure to rabies Deliberate use of Pasteur treatment as the foundation for maintenance immunity would not be recommended obviously because of the associated risk therefore this aspect has not been explored in detail However when treatment has been required because of exposure it is possible that profit may be taken from the misfortune and immunity maintained thereafter as suggested above Indeed proof that this may be done and guidance as to timing may be derived from the following data which bear more directly on managing the treatment of a new exposure

A total of 136 persons with histories of previous Pasteur treatment chiefly veterinarians but including some prisoner volunteers were persuaded to submit to a single inoculation of HEP Flury vaccine usually by the intradermal route and to one preinoculation and several postinoculation bleedings The results are summarized in TABLE 7 Definite response was demonstrated in 86 per cent of the total or excluding 9 persons for whom the tests were not determinative (see footnotes to TABLE 7) in 92 per cent (117 of 127) of those properly tested Furthermore of the 10 definite failures 6 were among 17 persons last treated 20 or more years previously Although some variation

TABLE 8

SUMMARY OF OBSERVATIONS AS TO PERSISTENCE OF NEUTRALIZING ANTIBODY AFTER PASTEUR TREATMENT

Number of treatments	Antibody level (titer)	Number of persons bled at stated years after last treatment									
		<1	1 2	3–4	5–6	7 8	9–11	12 15	16–20	21 26	35–40
Only 1	32+	1		3		2	3	1	3		
	8–31	2	3	2	2			2	1	1	
	4–7						1				
	<4 or P LT		7	1	1	3	1	3	4	1	1
	0	2	2	3	5	1	4	5	7	6	3
	All pos	3	10	6	3	5	5	6	8	2	1
	All	5	12	9	8	6	9	11	15	8	4
Two or more	32+	1	6	4	4	6	1	1			
	8–31		1	1			1	1	2		
	4 7						2				
	<4 or P LT					2	[illegible]	1	2		
	0					1	1		2	1	
	All pos	1	7	5	4	8	4	5	4	0	
	All	1	7	5	4	9	5	5	6	1	
Only 1	Per cent positive	60	84	67	38	84	56	55	53	25	25
		73			57			54		25	
Two or more		100	100	100	100	89	80	100	67	0	
		100			89			78		—	

P LT means positive in qualitative long incubation test in most such instances no quantitative tests were made Also <4 as in TABLE 4

TABLE 6

RELATION BETWEEN MAXIMUM ANTIBODY TITER AFTER PRIMARY COURSE AND AFTER BOOSTER OF HI P FLURY VACCINE AND PRESENCE OF ANTIBODY AT SUBSEQUENT INTERVALS

T ter f llow g	Ma mum titer (1)	Pr sen e f t body t i d at d intervals								
		5 Months			12 Mo ths			24 mo m th		
		T tal No	Numb with t ter 1		T tal N	Numb with t ter 1		T tal No	N mb w th t ter 1	
			<4	4 r >		<4	4 o >		<4	4 o >
Primary course	32+	2	1	1	2		2	4		4
	8–31	3	2	1	4	1	3	1	1	
	4–7	7	7		2	1	1	3	3	
	<4*	8	7	1	16	15	1	6	6	
Booster	32+				17	2	15	8	1	7
	8–31				1	1		0		
	4–7				4	4		2	1	1
	<4				1	1		1	1	

The symbol <4 in this case may include sera devoid of antibody since many were negative in 1 4 final dilution and not tested in lower dilution

a booster Second third and even fourth (in one instance only) boosters have been given but thus far to an insufficient number of persons to permit definition of the optimum spacing

As stated previously Pasteur treatment itself provides a foundation immunity upon which a program of maintenance immunity also may be built and which in any event carries important and long persisting implications

TABLE 7

SUMMARY OF ANTIBODY RESPONSES TO HFP FLURY BOOSTER INOCULA GIVEN TO PERSONS WITH HISTORIES OF PREVIOUS PASTEUR TREATMENT

Y s la t t eatm nt	N mb f pe s	P so po d gt		R ng f m m m po t t ‡ (1)
		N mbe	P nt	
unknown	5	4	80	>32 >1 024
35–40	4	1	25	128
20–26	13	9 (10)	69 (77)	<4–>4 100
15–19	21	20 (21)	95 (100)	<4–1 500
10–14	20	20	100	32–2 048
5–9	34	31 (33)	91 (97)	4–16 400
3 4	15	14 (15)	93 (100)	45–3 640
1–2	18	16 (17)	89 (94)	38–>4 096
<1	6	2 (5)	33 (83)	18 282
All	136	117 (126)	86 (93)	<4 16 400

Inocula usually intra lermal and in amounts ranging fr m 0 08 to 0 16 gm in 15 persons howe er intramuscular inocula of 2 0 gm were given

† Use of parentheses has same meaning as in TABLE 4

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with respect to the management of treatment on the occasion of any subsequent new exposure to rabies. Deliberate use of Pasteur treatment as the foundation for maintenance immunity would not be recommended, obviously, because of the associated risk; therefore this aspect has not been explored in detail. However, when treatment has been required because of exposure, it is possible that profit may be taken from the misfortune and immunity maintained thereafter as suggested above. Indeed, proof that this may be done and guidance as to timing may be derived from the following data, which bear more directly on managing the treatment of a new exposure.

A total of 136 persons with histories of previous Pasteur treatment, chiefly veterinarians but including some prisoner volunteers, were persuaded to submit to a single inoculation of HEP Flury vaccine, usually by the intradermal route, and to one preinoculation and several postinoculation bleedings. The results are summarized in TABLE 7. Definite response was demonstrated in 86 per cent of the total or, excluding 9 persons for whom the tests were not determinative (see footnotes to TABLE 7), in 92 per cent (117 of 127) of those properly tested. Furthermore, of the 10 definite failures, 6 were among 17 persons last treated 20 or more years previously. Although some variation

TABLE 8
SUMMARY OF OBSERVATIONS AS TO PERSISTENCE OF NEUTRALIZING ANTIBODY AFTER PASTEUR TREATMENT

Number of treatment courses	Antibody level (titer)	Number of persons observed at indicated time (years) after last treatment									
		<1	1-2	3-4	5-6	7-8	9-11	12-15	16-20	21-26	35-40
Only 1	32+	1		3		2	3	1	3		
	8-31	2	3	2	2			2	1	1	
	4-7						1				
	<4 or P LT		7	1	1	3	1	3	4	1	1
	0	2	2	3	5	1	4	5	7	6	3
	All pos	3	10	6	3	5	5	6	8	2	1
	All	5	12	9	8	6	9	11	15	8	4
Two or more	32+	1	6	4	4	6	1	1			
	8-31		1	1			1	1	2		
	4-7						2				
	<4 or P LT					2	2	1	2		
	0					1	1		2	1	
	All pos	1	7	5	4	8	4	5	4	0	
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		73			57			54		25	
Two or more		100	100	100	100	89	80	100	67	0	
		100			89			78		—	

P LT means positive in qualitative long incubation test; in most such instances no quantitative tests were made. Also <4 as in TABLE 4.

in response was noted, peak responses (usually by the fifteenth day) tended to surpass those following a full primary course of Pasteur treatment Although no sera were collected earlier than 15 days after the booster the common observation of maximum response titers in these 15 day sera indicates that the booster response usually was more rapid as well as greater than that to primary immunization It should be noted that 43 (or 32 per cent) of the group admitted having received 2 or more previous courses of treatment An analysis not here presented of the frequency and degree of response showed no differences as between those treated once and those given multiple treatments

Data as to duration of post treatment immunity became available as the prebooster sera were tested These are summarized separately in TABLE 8 for those previously treated only once and for those with histories of multiple courses In general remarkable antibody persistence was observed but the differences related to number of previous courses are of interest After but a single course more than one half of the subjects retained antibody through 20 years and nearly three fourths for 5 years However if 2 or more courses had been administered essentially 80 per cent had antibody for 20 years and all possessed it through 5 years From these data one might suggest the following (1) for maintenance immunity a booster dose (preferably of avian embryo vaccine) might be given at from 1 to 2 years after Pasteur treatment with subsequent boosters at 5 to 6 year intervals (or upon new exposure) and (2) *for new exposures in previously treated persons a single booster inoculum* (again preferably of avian embryo vaccine) is probably adequate if the most recent treatment occurred no longer than 20 years previously

Comment

The heart of the problem confronting the physician in the after the bite situation is the weighing of the evidence that true exposure has taken place against the risk known to be associated with conventional antirabic vaccination This has been delineated in classic manner by T F Sellers[31] in a paper aptly entitled Rabies the physician s dilemma which closed with the caution that for exposures other than actual bites the danger of treatment complications far exceeds that of either rabies or rabiphobia These words were published in 1948 At present although these words of caution must be borne in mind at least a little longer use of the avian embryo vaccines seems to ease the problem considerably especially in the case of those who already have undergone one course of Pasteur treatment Meanwhile to ensure the best possible response in the emergency situation efforts should continue to increase the antigenic potency of the embryo vaccines and to improve the methods for their use The other major problem posed by instances of severe exposure with probability of disease after a short incubation period has been greatly eased by the advent of antirabies hyperimmune serum However room for important improvements remains in the direction of increasing the potency and degree of purification in order to reduce the volume of the inoculation the average size of which is now 40 to 50 ml per adult and the likelihood of serum sickness reactions

For small but selected high risk segments of the population of the United States including veterinarians dog catchers and postmen the observations as to the long persistence after Pasteur treatment both of antibody and of conditioning to later booster inocula have special implications with respect to management of new exposures Often no inoculation may be needed (see TABLE 8 for guidance) and in any case one inoculum preferably of embryo vaccine should be sufficient For these same groups plus children resident in regions of high rabies endemicity the embryo vaccines would seem to offer a safe mechanism for laying a foundation immunity and for maintaining such a basic immunity including that induced by Pasteur treatment indefinitely by means of periodic booster doses The optimum spacing of such doses remains to be worked out but if the duration of postbooster seroimmunity is at all similar to that following Pasteur treatment in previously treated persons (again see TABLE 8) a possible schedule would have the foundation course followed within 1 or 2 years by the first booster and subsequent boosters coming every 5 or 6 years thereafter or as with tetanus toxoid upon the occasion of suspected exposure

A final note perhaps should be added The foregoing presentation and discussion have been based on the possibly debatable assumption that immunity to rabies and the readily demonstrable presence of neutralizing antibody are synonymous I am familiar with evidence (largely unpublished) that highly susceptible animals possessing antibody may succumb to challenge with rabies street virus and that other animals may resist it even though antibody cannot be demonstrated Because species susceptibility to rabies may vary significantly one cannot translate the experimental work in animals directly to man nor can one evaluate seroimmunity in man by the method of challenge As a consequence one must have a reasoned faith in the importance of neutralizing antibody the degree of faith obviously varying directly with the antibody titer

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ORNITHOSIS IN DOMESTIC FOWL NEWER FINDINGS IN TURKEYS

By John P Delaplane*

Department of Veterinary Microbiology Agricultural and Mechanical College of Texas and Texas Agricultural Experiment Station College Station Texas

The public health aspect of ornithosis infection in turkeys has been of interest since 1951 when Irons Sullivan and Rowen[1] published serologic and epidemiologic studies on an outbreak that occurred among poultry dressing plant workers in Giddings Texas in 1948 No additional infections involving such workers were recognized until November and December 1951 and in April 1952 when infections in poultry plant workers again were observed in the same plant that had experienced the 1948 outbreak reported by Irons *et al*[1, 2]

As a result of the outbreaks in 1951 and 1952 attempts were made to recover ornithosis virus from turkeys showing lower respiratory involvements because it was suspected that ornithosis might be masked by the lower form of infectious sinusitis In 1952 Boney *et al*[3] isolated an agent from turkeys that was later identified as ornithosis virus

Much interest was stimulated in ornithosis infection in turkeys in 1952 when a number of laboratory workers inadvertently became infected with ornithosis of turkey origin thus demonstrating the high pathogenicity of this agent for man No further outbreaks of ornithosis were recognized in turkeys or processing plant workers in Texas until April May and June of 1954 when approximately 200 nonfatal cases occurred in five processing plants in Texas A number of isolations of virus were made from turkey flocks in that state by Pate Boney and Delaplane[4]

Following recognition of the disease in Texas in 1952 and 1954 infections traceable to turkeys have been reported from New Jersey[5] and Oregon[6, 7] According to Andrews[8] ornithosis virus has been isolated from turkeys in seven states

Since 1955 a comprehensive research program on ornithosis as it affects turkeys has been under way at the Texas Agricultural Experiment Station Financial support for the research has been provided by the Department of Agriculture Agricultural Research Service Washington D C the Division of Research Grants National Institutes of Health Bethesda Md American Cyanamid Co Research Division Pearl River N Y Merck & Co Rahway N J and Hess and Clark Inc Ashland Ohio

The following phases of work have received special emphasis (1) the gross lesions of the disease in turkeys from one through twenty four weeks (2) the effect of chlortetracycline in the feed on the course of the infection in turkeys on a prophylactic basis and as therapy following infection (3) the possibility

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* Deceased

of egg transmission of the virus to the newly hatched poult (4) serology studies using the indirect complement fixation and other serologic tests to study sera from turkeys under field and experimental conditions (5) potential reservoirs of virus in nature through the screening of representative free flying birds and rodents from six turkey farms under study

More recently specimens of wading birds have been obtained from the rookeries along the coast of the Gulf of Mexico

Gross Lesions

Extensive studies of the gross lesions of ornithosis in turkeys by Davis *et al*[9] confirmed the preliminary observations of Boney *et al*[3] and of Pate Boney and Delaplane[4] and showed that the major lesions consisted of a high incidence of caseofibrinous pericarditis perihepatitis occurring as a fibrinous coating or plastic exudate biliary stasis caseofibrinous peritonitis caseofibrinous exudates on the air sacs pneumonia and splenomegaly In experimental infections the mortality[9] varied with the age of the turkey It was almost 100 per cent in 1 week old poults but declined to approximately 20 to 25 per cent in the 12 to 24 week old birds The incubation period varied from 4 days in young poults to 7 days in older turkeys

Microscopic studies of the lesions in tissues collected from the gross pathology phase of the study are in progress

In limited studies[9] evidence of airborne transmission of ornithosis in turkeys could not be proved

Prophylaxis and Therapy

Prophylactic therapy using chlortetracycline at the rate of 100 200 400 600 and 800 gm per ton of feed as reported by Davis and Delaplane[9,11] showed that all levels were effective in preventing mortality in 3 week old experimentally infected poults over a 2 week period of observation Extensive symptoms and lesions were observed in the inoculated poults on the 100-gm level of antibiotic virus was easily recovered from these poults as well as from the untreated infected controls Only slight lesions were observed in the poults receiving 200 gm of antibiotic per ton of feed and virus was recovered only from the poults with lesions No symptoms or lesions were observed in the poults receiving 400 600 and 800 gm of antibiotic per ton of feed and virus was not recovered from any of these poults

Recently Davis and Watkins[12] studied furazolidone at the rate of 100 200 and 300 gm per ton of feed in the prevention of ornithosis using similar procedures as for the chlortetracycline studies and they found it to be ineffective

The therapy studies of Davis and Delaplane[1] using chlortetracycline at the rate of 100 200 400 and 800 gm per ton of feed for 2 weeks in 3 week-old infected turkeys showed that such levels did not result in the elimination of virus When similar treatment was given for 3 weeks 100 gm was ineffective but 200 gm and more supressed the infection

Infected adult turkeys receiving 200 and 400 gm of chlortetracycline per ton of feed for 2 or 3 weeks were able to overcome the virus Ornithosis virus

was recovered from untreated infected adults 24 days after inoculation but not after 31 days.

In the fall of 1956 Davis, Watkins, and Delaplane[11] reported on the treatment of naturally occurring ornithosis in a farm flock. Attempted therapeutic measures by the owner had not been successful. Later supervised therapy using 200 gm. chlortetracycline per ton of feed for 3 weeks suppressed the infection. The flock was processed at a federally inspected plant and ornithosis virus was not recovered. One hundred sick turkeys were removed from the farm and placed under study at the Texas Agricultural Experiment Station Research Farm and given 200 gm. of chlortetracycline per ton of feed for 3 weeks. Virus was present in the experimental flock at the end of 2 weeks but not after 3 weeks.

Egg Transmission

Preliminary studies on the role of turkey eggs in the transmission of ornithosis by Davis and Delaplane[10] revealed no evidence of transmission. More extensive studies were undertaken by these investigators[14] and no virus was recovered from 2954 eggs laid by breeder turkeys experimentally infected with the Jo strain of ornithosis, although 300 of the eggs were laid during the time at which virus could be recovered from the blood stream of representative birds. When fresh turkey eggs were inoculated with ornithosis virus and incubated, the virus was not recovered after the ninth day of incubation. When 7 day-old embryonating turkey eggs were inoculated, the embryo mortality rate was high and the virus was not recovered beyond the sixteenth day in the few that survived.

Further studies showed that the virus was not recovered from eggs laid by hens in the viremic stage of ornithosis when isolation attempts were made on freshly laid and 24-hour incubated eggs. Ornithosis virus failed to live longer than 3 days on turkey egg shells or longer than 4 days on cotton swabs under egg incubator conditions.

Serologic Findings

Since 1955, turkey serum samples obtained from the pullorum testing laboratories representing Texas turkey breeding flocks have been tested using the indirect complement fixation (ICF) test. Approximately 50 per cent of the flocks showed reactions at 1:2, 1:4, 1:8, and 1:16, but the history of such flocks did not indicate the presence of ornithosis infection. Recently Neal and Davis[17] compared the efficiency of the direct complement fixation (DCF) of Benedict[18, 19] and an agglutination test with the ICF tests on the same serum samples of experimental and farm source of flocks. The agglutination test as used was of no value; however, there was about 85 per cent correlation between the ICF and the Benedict DCF test results.

During and immediately following the age of maturity of one of the experimental groups on study, approximately 30 per cent of these known laboratory reared ornithosis free turkeys showed reactions much like those experienced under farm flock conditions. Later representative birds from the ornithosis-

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Discussion of the Paper

OSCAR SUSSMAN (*Bureau of Veterinary Public Health, State of New Jersey Department of Health Trenton N J*) Raymond Fagan and I have recently completed a study based on the treatment of known infected turkeys with 160 mg of oxytetracycline given intramuscularly and into the hanging wattles of the birds Our report is not yet ready for publication, but I can state that the injection of this amount of antibiotic into birds in the midst of an epidemic resulted in isolation of no virus in birds killed 3 weeks 4 to 7 weeks and as long as 8 weeks thereafter Spleens were examined and isolation attempts were made on all of the flock after treatment Virus could be obtained only from birds that had not received treatment The material used was an intramuscular compound diluted with physiological saline solution to 80 mg/cc The mineral oil suspension* was one of 80 mg/cc It should be noted that the intramuscular material discolored the injected area of the musculature and it was found that injection into the wattle appeared to be as effective and did not injure any of the salable portion of the bird

* Supplied by Chas Pfizer & Co Inc Brooklyn N Y

NEWER METHODS FOR DETECTION OF AVIAN ORNITHOSIS*

By Albert A. Benedict† and Clarence McFarland†
Department of Preventive Medicine and Public Health, University of Texas Medical Branch, Galveston, Texas

The increased awareness of the public health importance of turkey ornithosis has stimulated re-examination of the methods used for detection of this infection in domestic fowls. The detection of exposure to ornithosis may be considered to have 3 principal objectives: (1) for diagnostic purposes, (2) to explore the epidemiological facets of the infection chain, which ultimately will lead to (3) the development of control measures. Obviously, definitive diagnosis is made by isolation and identification of the agent and, as evidenced by recent epizootics in turkeys, virus isolation is made frequently and easily. In severe epizootics the appearance of high titer circulating antibody in a large number of birds can be considered as direct evidence of infection. Diagnosis is also justified by a significant rise in titer when paired serum samples are available.

The significance of the presence of a few serologic reactors with low antibody titers among large flocks is not clear. There exist several possibilities that might explain these situations, such as (1) the sign of initial ornithosis activity in a flock, (2) the residue of ornithosis infection that was originally manifested with or without clinical ornithosis, (3) the presence of a cross-reacting antibody to an organism similar to the bacterium isolated by Volkert and Matthiesen,[1] and (4) the presence of unknown nonspecific serologic reactions. The control of ornithosis will be aided by the accurate interpretation of the significance of these reactions.

Until recently the serologic technique used exclusively for revealing circulating antibody in domestic fowl has been the indirect complement fixation test (ICF) developed by Karrer, Meyer and Eddie. The complement fixation (CF) test was not applied for such studies since, among other avian sera, Rice[2] reported the failure of antipullorum turkey sera to fix complement (C′) with *S. pullorum* and Eddie and Francis[4] observed fixation at only low dilutions with a small number of turkey anti-ornithosis sera. The presence of a specific inhibitor, presumably an incomplete type antibody, was demonstrated in avian noncomplement fixing sera[3,4] and thus the ICF was devised for detection of such antibodies.[3,5,6]

It was generally believed, therefore, that turkeys and chickens do not produce the usual CF antibodies until recent studies showed that detection of turkey CF antibody depended partially upon the type of antigen employed.[7] Moreover, in 1924 Bushnell and Hudson[8] reported that chicken antipullorum sera fixed C′ with homologous antigen, but that this property was destroyed

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by heat inactivation of the sera This early observation was reinvestigated by our laboratory and by Brumfield and Pomeroy,[9] and it revealed that the CF ability of turkey and chicken ornithosis antibody was destroyed by heat inactivation of the sera if virus particle antigens were employed

A variety of serologic tests have been made available however only the ICF and the direct complement fixation test (DCF) will be considered in this report In addition the intradermal allergic test was developed as an ancillary epidemiological tool[10, 11] At present there is an urgent need to evaluate and to compare the various methods that have been proposed so that a standard technique may be developed With this in view this report presents the development and evaluation of the DCF and allergic tests

Direct Complement Fixation Using Heat Inactivated Serum

Technique We have described the extraction of a soluble psittacosis CF antigen with a detergent[12] this antigen fixed C consistently with turkey antiornithosis sera[7] Fixation occurred with sera that had been inactivated by heating either at 56° C for 30 min or at 60° C for 20 min For this test 4 units of the detergent extracted antigen were used In studies that compared antigen activities complement was titrated in the presence of antigens Preliminary incubation was carried out at 37° C for 75 min and after the addition of sensitized sheep erythrocytes a secondary incubation at 37 C for 30 min was done The method used in performing the ICF test was similar to that described by Karrer Meyer and Eddie Titers represent the reciprocal of the highest dilution of serum showing either a 1+ or 2+ reaction in the ICF (partial inhibition) or a 4+ reaction (complete fixation) in the DCF

Comparison of the DCF and ICF methods A comparison of these techniques was made with naturally infected and experimentally infected turkeys Forty seven turkeys were selected at random from a flock that had undergone a severe epizootic several months prior to bleeding TABLE 1 shows that 41 sera in this group (A) were positive by both the ICF and DCF and that 3 sera were positive in low dilutions in the DCF and negative in the ICF Also shown in TABLE 1 are results with sera from 2 flocks (B and C) in which there was no evidence of infection at the time of bleeding One serum from flock B was negative by the ICF and had a DCF titer of 1 4 Sera obtained from 12 turkeys that had undergone a natural infection 18 months prior to the test were all positive to the DCF and only 5 sera had positive ICF titers A direct correlation analysis on the basis of the log distribution of the ICF and DCF titers of group A was found to be 0 72 which was significant at the 1 per cent level Although there is no direct evidence as yet that the same antibody was being measured by the 2 tests the DCF titers correlated well with the ICF titers of these sera

In search of a reliable simple and economical serologic technique for survey purposes Neal and Davis[13] compared the ICF DCF (detergent antigen) and a macro [illegible]tination test[14] that employed stained elementary bodies as antige[illegible] and DCF agreed in 85 1 per cent of 923 turkey era tested Th[illegible] similar to the findings shown in TABLE 1 in which the 2

TABLE 1
COMPARISON OF THE DIRECT AND INDIRECT COMPLEMENT FIXATION TESTS FOR THE DETECTION OF TURKEY ORNITHOSIS

Group	History	Number of turkeys tested	DCF* pos ICF† pos.	DCF pos ICF neg	DCF neg ICF pos	DCF neg ICF neg	Per cent positive		Per cent agreement
							DCF	ICF	
A	Natural infection for 3 months	47	41	3	0	3	93.6	87.3	93.6
B	No evidence of infection	61	1	1	0	59	3.2	1.6	98.3
C	No evidence of infection	50	2	0	0	48	4.0	4.0	100.0
D	Natural infection 18 months before	12	7	5	0	0	100.0	58.3	58.3
E	Experimentally infected 2 months	21	15	1	3	2	76.1	85.7	80.9
F	Experimentally infected 3 months	12	8	0	0	4	66.6	66.6	100.0
Total		203	74	10	3	116	41.3	37.9	88.5

* Direct complement fixation
† Indirect complement fixation

tests agree in 88.5 per cent of 203 turkey sera tested. On the other hand the correlation between the results of the agglutination and ICF tests was 42.3 per cent and between the agglutination and DCF tests the correlation was 40.4 per cent. Many nonspecific reactions were given by the agglutination test by lower serum dilutions, an observation that we had also made relative to an ornithosis precipitin reaction with turkey and chicken sera.[16] On the basis of these comparisons further study with this DCF test was considered justified.[13]

Onset of DCF antibody. It was felt that if incomplete CF antibodies were formed in turkey sera they might have been predominant during the early stages of the infection. On the contrary, however, positive DCTs were demonstrated one to two weeks following infection (TABLE 2). The earliest appearance of the DCF antibodies paralleled the appearance of ICF antibodies.

Activity of antigens. Numerous attempts were made to yield CF reactions with heated turkey sera and antigens made from either yolk sac or allantoic fluid virus suspensions. For example, a heated phenolized psittacosis (strain 6 BC) yolk sac antigen, a viable concentrated meningopneumonitis virus suspension and the detergent extract were each titrated against 4 units each of rabbit and turkey antipsittacosis sera. The soluble antigen had the same titer when tested against rabbit and turkey sera (TABLE 3). The viral suspensions were considerably less active when titrated with turkey antiserum than with rabbit antiserum. It was obvious that viral suspensions were unsuitable as antigens with heat inactivated sera. Direct fixation with turkey sera would not usually be obtained if the virus suspension antigens were standardized with nonavian or pigeon sera and if then the usual 4 units of antigen were used in the direct test with turkey sera. When tested with rabbit antiserum, 4 units of the allantoic fluid and yolk sac antigens shown in TABLE 3 represent

by heat inactivation of the sera. This early observation was reinvestigated by our laboratory and by Brumfield and Pomeroy[9] and it revealed that the CF ability of turkey and chicken ornithosis antibody was destroyed by heat inactivation of the sera if virus particle antigens were employed.

A variety of serologic tests have been made available; however, only the ICF and the direct complement fixation test (DCF) will be considered in this report. In addition, the intradermal allergic test was developed as an ancillary epidemiological tool.[10, 11] At present there is an urgent need to evaluate and to compare the various methods that have been proposed so that a standard technique may be developed. With this in view, this report presents the development and evaluation of the DCF and allergic tests.

Direct Complement Fixation Using Heat Inactivated Serum

Technique. We have described the extraction of a soluble psittacosis CF antigen with a detergent;[12] this antigen fixed C' consistently with turkey antiornithosis sera.[7] Fixation occurred with sera that had been inactivated by heating either at 56° C. for 30 min. or at 60° C. for 20 min. For this test 4 units of the detergent extracted antigen were used. In studies that compared antigen activities complement was titrated in the presence of antigens. Preliminary incubation was carried out at 37° C. for 75 min. and, after the addition of sensitized sheep erythrocytes, a secondary incubation at 37° C. for 30 min. was done. The method used in performing the ICF test was similar to that described by Karrer, Meyer and Eddie. Titers represent the reciprocal of the highest dilution of serum showing either a 1+ or 2+ reaction in the ICF (partial inhibition) or a 4+ reaction (complete fixation) in the DCF.

Comparison of the DCF and ICF methods. A comparison of these techniques was made with naturally infected and experimentally infected turkeys. Forty-seven turkeys were selected at random from a flock that had undergone a severe epizootic several months prior to bleeding. TABLE 1 shows that 41 sera in this group (A) were positive by both the ICF and DCF, and that 3 sera were positive in low dilutions in the DCF and negative in the ICF. Also shown in TABLE 1 are results with sera from 2 flocks (B and C) in which there was no evidence of infection at the time of bleeding. One serum from flock B was negative by the ICF and had a DCF titer of 1:4. Sera obtained from 12 turkeys that had undergone a natural infection 18 months prior to the test were all positive to the DCF, and only 5 sera had positive ICF titers. A direct correlation analysis on the basis of the log distribution of the ICF and DCF titers of group A was found to be 0.72, which was significant at the 1 per cent level. Although there is no direct evidence as yet that the same antibody was being measured by the 2 tests, the DCF titers correlated well with the ICF titers of these sera.

In search of a reliable, simple, and economical serologic technique for survey purposes, Neal and Davis[13] compared the ICF, DCF (detergent antigen), and a macroscopic agglutination test[14] that employed stained elementary bodies as antigen. The ICF and DCF agreed in 85.1 per cent of 923 turkey sera tested. These results were similar to the findings shown in TABLE 1, in which the 2

TABLE 1
COMPARISON OF THE DIRECT AND INDIRECT COMPLEMENT FIXATION TESTS FOR THE DETECTION OF TURKEY ORNITHOSIS

Group	History	Number of turkeys tested	DCF* pos ICF† pos	DCF pos ICF neg	DCF neg ICF pos	DCF neg ICF neg	Per cent positive DCF	Per cent positive ICF	Per cent agreement
A	Natural infection for 3 months	47	41	3	0	3	93.6	87.3	93.6
B	No evidence of infection	61	1	1	0	59	3.2	1.6	98.3
C	No evidence of infection	50	2	0	0	48	4.0	4.0	100.0
D	Natural infection 18 months before	12	7	5	0	0	100.0	58.3	58.3
E	Experimentally infected 2 months	21	15	1	3	2	76.1	85.7	80.9
F	Experimentally infected 3 months	12	8	0	0	4	66.6	66.6	100.0
Total		203	74	10	3	116	41.3	37.9	88.5

* Direct complement fixation
† Indirect complement fixation

tests agree in 88.5 per cent of 203 turkey sera tested. On the other hand, the correlation between the results of the agglutination and ICF tests was 42.3 per cent and between the agglutination and DCF tests the correlation was 40.4 per cent. Many nonspecific reactions were given by the agglutination test by lower serum dilutions, an observation that we had also made relative to an ornithosis precipitin reaction with turkey and chicken sera.[15] On the basis of these comparisons further study with this DCF test was considered justified.[12]

Onset of DCF antibody. It was felt that if incomplete CF antibodies were formed in turkey sera, they might have been predominant during the early stages of the infection. On the contrary, however, positive DCFs were demonstrated one to two weeks following infection (TABLE 2). The earliest appearance of the DCF antibodies paralleled the appearance of ICF antibodies.

Activity of antigens. Numerous attempts were made to yield CF reactions with heated turkey sera and antigens made from either yolk sac or allantoic fluid virus suspensions. For example, a heated phenolized psittacosis (strain 6 BC) yolk sac antigen, a viable concentrated meningopneumonitis virus suspension, and the detergent extract were each titrated against 4 units each of rabbit and turkey antipsittacosis sera. The soluble antigen had the same titer when tested against rabbit and turkey sera (TABLE 3). The viral suspensions were considerably less active when titrated with turkey antiserum than with rabbit antiserum. It was obvious that viral suspensions were unsuitable as antigens with heat-inactivated sera. Direct fixation with turkey sera would not usually be obtained if the virus suspension antigens were standardized with nonavian or pigeon sera and if then the usual 4 units of antigen were used in the direct test with turkey sera. When tested with rabbit antiserum, 4 units of the allantoic fluid and yolk sac antigens shown in TABLE 3 represent

TABLE 2
THE APPEARANCE OF COMPLEMENT FIXING ANTIBODIES IN EXPERIMENTALLY INFECTED TURKEYS

Turkey	Days after infection				
	0	8	10	14	108
1	0	2 (3+)		64	
2	0	16		32	
3	0	2 (3+)		8	
4	0	2		64	
5	0	4		8	
1977	0		64		128
1852	0		128		<4
1915	0		128		32
1849	0		256		<4
1984	0		256		<4
1943	0		128		4
1962	0		512		64

Negative at 1:2 dilution

titers of 32 and 64, respectively, these concentrations were insufficient to fix C' with turkey sera.

The detergent extract apparently consisted of an antigen that differed either quantitatively or qualitatively from that found in the particle suspensions. To test this hypothesis the antigen was purified by acid precipitation and fractionated with phenol.[1] Phenol fractionation produces soluble and insoluble CF antigens that differ chemically. TABLE 3 summarizes the results of titrating these fractions against 4 units each of guinea pig, human, and turkey

TABLE 3
COMPLEMENT FIXING ACTIVITY OF ANTIGENS WHEN TESTED WITH MAMMALIAN AND TURKEY ANTIPSITTACOSIS SERA

Antigen	Psittacosis antiserum			
	Guinea pig	Human	Rabbit	Turkey
Living meningopneumonitis allantoic fluid suspension			128†	16
Killed psittacosis yolk sac suspension			256	8
Crude detergent extract			64	64
Phenol insoluble fraction of detergent extract				
1	16			16
2	16			16
3		32		32
Phenol soluble fraction of detergent extract				
1	8			0
2	32			0
3		16		0

Four units of each antiserum
† CF titer of antigens

antisera The phenol soluble fraction reacted similarly to the particle antigens and failed to fix C with heated turkey antibody whereas the phenol insoluble antigen had the same titer when tested against turkey human and guinea pig sera Possibly this latter fraction represents a subsurface antigen that was released by extraction However this hypothesis is not yet tenable in view of the fact that the absorption of heated turkey sera with virus particles also absorbs the antibody to the phenol insoluble fraction

When we employed the detergent extracted antigen our attempts to obtain direct fixation of C with heat inactivated chicken antisera met with repeated failures Nevertheless this antigen combined with heated chicken antibody and gave an ICF reaction

Direct Complement Fixation Using Unheated Serum

Techniques As previously mentioned Bushnell and Hudson[8] observed that chicken and turkey antibody failed to fix C with heat inactivated sera However CF tests with unheated sera are unsuitable because of strong anticomplementary (AC) properties Brumfield and Pomeroy[9] proposed a method in which the C was increased to overcome the AC activity of unheated fowl sera In this test yolk sac antigens were employed and complement was titrated in the presence of 10 unheated sera diluted 1 8 Sera to be tested were diluted 1 8 to minimize AC effects and lytic action due to the fowl C There was agreement between the ICF and DCF in more than 94 per cent of 125 turkey sera tested

A second method might be one that selectively removes the AC substances from unheated sera thereby avoiding use of excessive amounts of C and possibly permitting tests with lower serum dilutions With this in mind the following procedures were attempted to remove chicken and turkey sera AC activity (1) chloroform extraction (2) hypertonization with NaCl[16] (3) acid precipitation[17] (4) adsorption with activated charcoal (5) adsorption with a mouse liver preparation[18] and (6) adsorption with kaolin Kaolin adsorption proved to be the most effective Complete details of the procedure will be given elsewhere and are given in brief form as follows A 1 2 dilution of 1 2 ml of antiserum is adsorbed with 40 to 50 mg of kaolin for 60 min The kaolin is removed by centrifugation at 1500 rpm for 5 min Under these conditions AC activity was effectively removed (TABLE 4) Also the serum was decomplemented and prozones were avoided Antibody was rarely adsorbed from turkey sera with 40 to 50 mg of kaolin but some chicken sera showed decreased ICF and DCF titers following adsorption Both DCF and ICF chicken antibodies were completely removed from 1 2 ml of a 1 4 dilution of serum adsorbed with the above quantities of kaolin Nikkila and Oker Blom[19] observed however that adsorption of human serum with clay minerals resulted in partially selective removal of lipids without loss of gamma globulin and Clarke and Casals[20] successfully used the clay mineral bentonite to remove hemagglutination inhibitor from antisera

Effect of storage on DCF antibody The ability of the detergent extracted antigen to fix C with heated turkey serum and the inability of the particle antigens to exert the same degree of activity suggested that these antigens

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	0	8	10	14	108
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2	0	16		32	
3	0	2 (3+)		8	
4	0	2		64	
5	0	4		8	
1977	0		64		128
1857	0		128		<4
1915	0		128		32
1849	0		256		<4
1984	0		256		<4
1943	0		128		4
1962	0		512		64

* Negative at 1:2 dilution

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Phenol insoluble fraction of detergent extract				
1	16			16
2	16			16
3		32		32
Phenol soluble fraction of detergent extract				
1	8			0
2	32			0
3		16		0

* Four units of each antiserum
† CF titer of antigens

TABLE 6

COMPLEMENT FIXATION TITERS OF UNHEATED KAOLIN ADSORBED TURKEY ANTISERA STORED AT 4 C

Turkey	Days stored									
	0		4		5		10		13	
	D*	YS†	D	YS	D	YS	D	YS	D	YS
12	16	0‡								
149	64	16								
42	8	0								
14	2	0								
109	8	2			8	0				
101	32	8			32	2				
29	32	2			32	4				
127	16	8			16	0				
16	32	8			32	16				
49	8	0			8	0				
148	16	8	16	2	16	0				
167	16	4					16	0		
13	16	2					16	0		
106	32	4					32	0		
155	(−)	4					<64	0		
29	64*	8					32	0		
46	16	4							16	0
8	16	4							16	4
28	8	2							4	0
9	4	(−)							2	0
27	16	2							16	0

Symbol (−) not tested
* Detergent antigen
† Yolk sac antigen
‡ Less than 1 2

presents the results of titrating these unheated kaolin adsorbed sera The titers were lower with the yolk sac antigen than with the soluble antigen and they decreased following refrigeration for 13 days Studies with turkey sera are being continued with only the soluble antigen since CF antibodies of unheated turkey sera are potentially labile to the particle antigens Unheated chicken antibody however loses the ability to fix C following storage at 4 C to both the soluble and particle antigens

Detection of chicken DCF antibody An interesting study performed by V T Briggs in our laboratory showed good correlation between the kaolin treated serum method and the ICF test To simulate a natural infection by the oral route the following experiment was performed Fifteen white leghorn chickens were infected with psittacosis virus (Texas turkey) Beginning 48 hours after infection feces were collected daily from these donors for 25 days Collections were pooled added to 200 cc of distilled water and homogenized in a Waring Blendor and a 1 4 dilution was made Forced feeding of this suspension to recipient chickens was accomplished by introducing a blunted pipette directly into the esophagus thereby passing the tracheal opening All daily feedings were made using stock inocula prepared from feces passed

TABLE 4
EFFECT OF CONCENTRATION OF KAOLIN AND TIME OF ADSORPTION ON REMOVAL OF ANTICOMPLEMENTARY ACTIVITY FROM CHICKEN SERUM

Mg kaolin	Time of adsorption in minutes			
	15	30	45	60
10	4	4	4	4
20	4	4	4	4
30	(−)	3	4	4
40	4	4	3	±
50	3	±	0	0

Anticomplementary activity of 1:2 dilution of serum.

might also have different activities with unheated sera. Turkey sera that had been stored at −20° C for 11 months and had been repeatedly frozen and thawed during this time were titrated (unheated and adsorbed with kaolin) with 4 units each of the detergent antigen and a yolk sac antigen. The serum titers obtained with the yolk sac antigen were significantly lower, and the titers obtained with the soluble antigen were the same as titers obtained when tested 11 months before by the heat inactivation method. Upon further storage of these sera at 4° C the titers continued to drop when tested with the yolk sac antigen.

Fresh turkey antisera had the same titers to the 2 antigens (TABLE 5). Following storage at 4° C for 4 days these sera showed decreased titers when tested with the yolk sac antigen (TABLE 5). According to these results and to those mentioned by Brumfield and Pomeroy,[9] caution must be exercised when using the viral suspension antigens for the testing of unheated fowl sera that had not been frozen soon after procurement. This fact was further borne out by testing turkey sera that had been mailed to us unrefrigerated. TABLE 6

TABLE 5
COMPLEMENT FIXATION TITERS OF UNHEATED KAOLIN ADSORBED TURKEY ANTISERA STORED AT 4° C

Turkey	Days stored			
	0		4	
	D	YS†	D	YS
1849	256‡	256	256	256
1852	128	128	128	<32
1984	256	256	256	32
1962	256	256	512	<32
1977	64	64	32	16
1406	0	0	0	0

Detergent antigen.
† Yolk sac suspension.
‡ CF titer of sera.

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	D	YS†	D	YS	D	YS	D	YS	D	YS
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149	64	16								
42	8	0								
14	2	0								
109	8	2			8	0				
101	32	8			32	2				
29	32	2			32	4				
127	16	8			16	0				
16	32	8			32	16				
49	8	0			8	0				
148	16	8	16	2	16	0				
167	16	4					16	0		
13	16	2					16	0		
106	32	4					32	0		
155	(–)	4					<64	0		
29	64*	8					32	0		
46	16	4							16	0
8	16	4							16	4
28	8	2							4	0
9	4	(–)							2	0
27	16	2							16	0

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* Anticomplementary activity of 1:2 dilution of serum

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140	64	16								
42	8	0								
14	2	0								
109	8	2			8	0				
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127	16	8			16	0				
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148	16	8	16	2	16	0				
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TABLE 7

COMPARISON OF INDIRECT AND DIRECT COMPLEMENT FIXING ANTIBODY RESPONSES IN ORALLY INFECTED CHICKENS

No. days donors infected	Chick	Titer day following first feeding of test recipients								No. pos
		14		21		28		40		
		DCF	ICF†	DCF	ICF	DCF	ICF	DCF	ICF	
2–6	1	16	16	4	8	4	16	4	8	3/4
	2	4	4	0	8	8	8	4	16	
	3	2	0	8	8	16	8	16	8	
	4	0	0	0	0	0	0	0	0	
	5 Cont	0	0	0	0	0	0	0	0	
7–11	6	0	0	16	32	0	2	sacrificed		3/5
	7	16	16	32	64	16	8	sacrificed		
	8	0	0	0	0	0	0	0	0	
	9	0	0	0	0	8	8	16	4	
	10	0	0	0	0	0	0	0	0	
	11 Cont	0	0	0	0	0	0	0	0	
12–16	12	0	0	0	(–)	0	0	0	0	1/5
	13	0	0	0	(–)	0	0	0	0	
	14	32	64	32	(–)	8	32	sacrificed‡		
	15	0	0	0	(–)	0	0	0	0	
	16	0	0	0	(–)	0	0	0	0	
	17 Cont	0	0	0	(–)	0	0	0	0	
17–21	18	0	(–)	0	0	0	(–)	0	(–)	0/5
	19	0	(–)	0	0	0	(–)	0	(–)	
	20	0	(–)	0	0	0	(–)	0	(–)	
	21	0	(–)	0	0	0	(–)	0	(–)	
	22	0	(–)	0	0	0	(–)	0	(–)	
	23 Cont	0	(–)	0	0	0	(–)	0	(–)	
22–26	24	0	(–)	0	0	0	(–)	0	(–)	0/3
	25	0	(–)	0	0	0	(–)	0	(–)	
	26	0	(–)	0	0	0	(–)	0	(–)	
	27 Cont	0	(–)	0	0	0	(–)	0	(–)	

Symbol (–) not tested

Direct complement fixation test with unheated sera that had been adsorbed with kaolin

† Indirect complement fixation

‡ Ornithosis isolated from spleen

during the previous 24 hour period. Inocula prepared daily from donor feces passed on days 2 through 6 were fed (1 ml. per bird per day) to a group of recipients. Four other groups similarly treated received respectively feces passed by donors 7 to 11, 12 to 16, 17 to 21 and 22 to 26 days following infection. All birds were therefore given 5 daily feedings. Birds within a given group were housed in the same cage along with an unfed control bird. The birds were bled on the days following the first feeding as shown in TABLE 7. Sera were frozen within 24 hours after bleedings. All birds were serologically negative on the first day of feeding. The DCF and ICF tests were equally sensitive for detection of serologic reactors and the serologic response was interpreted as a sign of infection. Apparently the feces were most infective

2 to 11 days following infection of the donor birds 6 of 9 recipient birds produced antibody Chicken No 14 was sacrificed 29 days following first feeding and the spleen yielded ornithosis virus on mouse passage Three blind passages of the spleens and livers of chickens No 6 and No 7 failed to reveal virus Working with the bovine encephalomyelitis virus Matumoto *et al*[21] indicated that the serologic response might be as efficient as direct isolation procedures for the detection of virus in infective materials

The Intradermal Test

Although the tuberculin test for detection of fowl tuberculosis[22] has had limited usage the presence of ornithosis hypersensitivity in chickens that had been experimentally infected suggested that the skin test might serve as an added epidemiological method for rapid detection of endemic foci[10, 11, 23] Sensitivity was detected as early as four weeks and as late as one year following infection[11] Circulating antibody appeared earlier than cutaneous hyper sensitivity during the course of the infection however the serologic and skin



FIGURE 1 Positive dewlap allergic response in an experimentally infected turkey Twenty four hour reaction

TABLE 7

COMPARISON OF INDIRECT AND DIRECT COMPLEMENT FIXING ANTIBODY RESPONSES IN ORALLY INFECTED CHICKENS

No. days donor feces fed	Chick	Titer on day following first feeding of feces to recipients								No. pos
		14		21		28		40		
		DCF*	ICF†	DCF	ICF	DCF	ICF	DCF	ICF	
2–6	1	16	16	4	8	4	16	4	8	3/4
	2	4	4	0	8	8	8	4	16	
	3	2	0	8	8	16	8	16	8	
	4	0	0	0	0	0	0	0	0	
	5 Cont	0	0	0	0	0	0	0	0	
7–11	6	0	0	16	32	0	2	sacrificed		3/5
	7	16	16	32	64	16	8	sacrificed		
	8	0	0	0	0	0	0	0	0	
	9	0	0	0	0	8	8	16	4	
	10	0	0	0	0	0	0	0	0	
	11 Cont	0	0	0	0	0	0	0	0	
12–16	12	0	0	0	(−)	0	0	0	0	1/5
	13	0	0	0	(−)	0	0	0	0	
	14	32	64	32	(−)	8	32	sacrificed‡		
	15	0	0	0	(−)	0	0	0	0	
	16	0	0	0	(−)	0	0	0	0	
	17 Cont	0	0	0	(−)	0	0	0	0	
17–21	18	0	(−)	0	0	0	(−)	0	(−)	0/5
	19	0	(−)	0	0	0	(−)	0	(−)	
	20	0	(−)	0	0	0	(−)	0	(−)	
	21	0	(−)	0	0	0	(−)	0	(−)	
	22	0	(−)	0	0	0	(−)	0	(−)	
	23 Cont	0	(−)	0	0	0	(−)	0	(−)	
22–26	24	0	(−)	0	0	0	(−)	0	(−)	0/3
	25	0	(−)	0	0	0	(−)	0	(−)	
	26	0	(−)	0	0	0	(−)	0	(−)	
	27 Cont	0	(−)	0	0	0	(−)	0	(−)	

Symbol (−) not tested

* Direct complement fixation test with unheated sera that had been adsorbed with kaolin

† Indirect complement fixation

‡ Ornithosis isolated from spleen

during the previous 24 hour period. Inocula prepared daily from donor feces passed on days 2 through 6 were fed (1 ml. per bird per day) to a group of recipients. Four other groups similarly treated received respectively feces passed by donors 7 to 11, 12 to 16, 17 to 21, and 22 to 26 days following infection. All birds were therefore given 5 daily feedings. Birds within a given group were housed in the same cage along with an unfed control bird. The birds were bled on the days following the first feeding as shown in TABLE 7. Sera were frozen within 24 hours after bleedings. All birds were serologically negative on the first day of feeding. The DCF and ICF tests were equally sensitive for detection of serologic reactors and the serologic response was interpreted as a sign of infection. Apparently the feces were most infective

TABLE 8
DETECTION OF ALLERGIC AND SEROLOGIC REACTORS AMONG HEALTHY TURKEYS AND TURKEYS NATURALLY INFECTED WITH ORNITHOSIS

Ranch	History	Skin test result			Serologic result		
		No. tested	No. pos.	Per cent pos.	No. tested	No. pos.	Per cent pos.
SK_1	1 yr-olds natural infection 3 to 4 months	232	165	71	47	40	85
SK_2	2 yr-olds close contact with infected birds SK	34	12	35	(−)	(−)	(−)
SK	2 to 5 yr olds close contact with infected birds SK_1	53	6	11	52	4	8†
J	1 yr olds natural infection 3 to 4 months	255	196	78	190	157	83
H_1	1 yr olds healthy	82	0	0	(−)	(−)	(−)
H_2	1 yr olds healthy	(−)	(−)	(−)	109	5	5
R	1 yr olds healthy	242	0	0	170	19	11
A & M	8 to 10 months old healthy	110	1	0.9	98	0	0†
Colorado	14 wks old healthy	1049	0	0	1[illegible]0	0	0†
Total		2057	380	19	837	225	27

Symbol (−) not tested
Indirect complement fixation test
† Direct complement fixation test heat inactivated sera

The results of testing these birds for hypersensitivity and for the presence of serum antibody are summarized in TABLE 8. Seventy one and 78 per cent respectively of the 1 year-old SK and J flocks were skin reactors. The percentages of serologic reactors from these flocks were essentially the same as the allergic reactor tests. In contrast to the large number of reactors among the 1 year-old birds only 35 per cent of the 2 year old and 11 per cent of the 2 to 5 year old birds of the SK flock were skin reactors. Similarly only 8 per cent of the oldest birds were serologically positive. In the SK flock the breeder observed that the infection was not as severe and that the mortality rate was less in the toms than in the hens.[24] Such differences were not noted in the J flock. The number of skin reactors among the toms concurred with this observation. Of the 232 SK birds tested 24 were toms and only 3 or 12 2 per cent were positive. In the J flock (255 birds) 5 or 71 per cent of 7 toms were positive reactors.

Further suggesting the application of the intradermal test for epidemiological purposes it was noted by the breeder that during the epizootic the mortality rate among older birds on ranch SK was lower than among the younger birds. The small number of allergic and serologic reactors attest to this observation.

Flocks H and R although in close proximity to flocks SK and J showed no clinical ornithosis at the time of testing and no skin reactors were found. However of 170 birds from flock R tested for ICF antibody 19 showed titers of 1 2 or above. Among 109 birds tested from flock H 4 6 per cent were positive

test methods were equally sensitive for detection of reactors among birds infected for four weeks or longer To further evaluate the intradermal test we tested naturally infected turkeys under field conditions [23]

Technique In the early experiments intradermal inoculation of turkeys with 0 1 ml of antigen was made in the dewlap and on the lateral surface of the thigh The dewlap was finally selected as the inoculation site since reactions at this site were easier to judge and developed earlier than the thigh reaction The reactions were judged by palpation of the dewlap for edema 24 hours following skin test Positive dewlap reactions are shown in FIGURES 1 and 2 Since the antigen was more likely to yield false negative rather than false positive reactions detection of minimal edema was considered positive

Comparison of intradermal and serologic tests Turkeys were obtained from the following sources (1) normal broad breasted bronze (BBB) and Beltsville (BV) turkeys and BV turkeys that had been experimentally infected at the Agricultural and Mechanical College of Texas, College Station Texas (2) 1 to 5 year old BBB turkeys that had experienced an ornithosis epizootic in Oregon approximately 3 to 4 months prior to testing (flocks SK and J) (3) 2 flocks of 1 year old BBB turkeys (flocks H and R) from the same vicinity as SK and J flocks but without evidence of clinical ornithosis infection when tested and (4) 2 flocks of healthy 14 week old BBB turkeys located in east Colorado



FIGURE 2 Positive dewlap allergic response in a naturally infected turkey Twenty four hour reaction

TABLE 10
COMPARISON OF THE SKIN TEST AND SEROLOGIC METHODS FOR THE DETECTION OF ORNITHOSIS IN NATURALLY AND EXPERIMENTALLY INFECTED TURKEYS

Turkey	No. tested	ST* pos. sero† pos.	ST neg. sero pos.	ST pos. sero neg.	ST neg. sero neg.	Per cent reactors		Per cent both tests agreed
						ST	Sero	
Natural infection for 3 to 4 months SK flock	48	39	5	2	2	85	92‡	85
Natural infection for 3 to 4 months J flock	45	27	3	8	7	73	67§	76

* Skin test
† Serologic
‡ Complement fixation test, heat inactivated sera
§ Indirect complement fixation test

by the ICF test. It should be pointed out that the birds skin tested from flock H were considered prize birds and had been separated from the flock because of their robust appearance, whereas the serologically positive birds were from the culled flock. It was known that the sensitivity of the intradermal test was limited *during early infection*. *Studies have shown that allergic* sensitization developed later than circulating antibody.[11] Subsequent to this study, an ornithosislike agent was isolated from flock H, and the number of serum reactors from both H and R flocks had increased.[4] It would appear therefore that the infection in these flocks was initiated shortly prior to the time of skin testing and that serum antibody appeared prior to the development of allergy. In such a situation the skin test might yield supporting data on the initiation of ornithosis activity in this flock. It should be emphasized that the intradermal test is not suitable for diagnostic purposes but is suitable for mass surveys to determine the extent of past infections.

The ICF, DCF (heat inactivated sera), and skin test methods were compared in 48 randomly selected birds from flock SK; the results given in TABLE 9 show that the allergic test compared favorably with the two serologic techniques. The results further summarized in TABLE 10 show that the serologic and skin tests agreed in 85 and 76 per cent of the naturally infected birds.

TABLE 11
ABILITY OF DIFFERENT ANTIGENS TO FIX COMPLEMENT WITH TURKEY AND CHICKEN ANTI-ORNITHOSIS SERA

Antigen	Turkey antiserum		Chicken antiserum	
	Unheated	Heated	Unheated	Heated
Virus suspensions	+	−	+	−
Detergent extract	+	+	+	−
Phenol-soluble fraction of extract	+	−	+	−
Phenol insoluble fraction of extract	+	+	+	?

TABLE 9
COMPARISON OF THE INTRADERMAL AND SEROLOGIC REACTIONS IN TURKEYS NATURALLY INFECTED WITH ORNITHOSIS

Turkey	Titer of sera		Skin test
	Direct complement fixation	Indirect complement fixation	
3	16	32	neg
12	4	0	neg
20	0	0	neg
23	8	4	neg
35	256	128	neg
49	0	0	neg
50	128	128	neg
1	8	256	+
2	32	128	+
4	128	256	+
5	(−)	0	+
66	256	256	+
7	16	(−)	+
8	32	64	+
9	256	32	+
10	256	64	+
11	32	8	+
13	128	128	+
14	128	256	+
15	64	8	+
16	256	128	+
17	256	128	+
18	32	16	+
19	128	32	+
21	64	128	+
22	64	128	+
24	128	32	+
25	8	16	+
26	256	256	+
27	8	8	+
28	32	32	+
29	64	64	+
30	16	4	+
31	8	8	+
32	64	8	+
33	512	256	+
34	16	4	+
36	4	0	+
37	128	32	+
38	128	64	+
39	128	16	+
40	16	16	+
41	16	0	+
42	*0*	*0*	+
43	64	256	+
45	16	4	+
47	16	8	+
48	8	256	+
Per cent positive	92	85	85

Symbol (−) not tested
Heat inactivated sera

3 Rice C E 1947 Can J Comp Med Vet Sci 11 236
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This was in good agreement with the 80 per cent correlation previously obtained with experimentally infected chickens[11]

Conclusions

The results of the experiments with turkey and chicken antibody indicate that their abilities to fix C′ with homogolous antigen differ These two species probably cannot be grouped as producing qualitatively similar CF antibodies The reactive group (or groups) on the antibody molecules associated with fixation of C′ are apparently heat labile but the degree of lability is different for turkey and chicken antibody as evidenced by the fixation of C with heated turkey serum and the soluble antigen Turkey and chicken antibody are dissimilar in at least this one respect A summary of these reactions is given in TABLE 11 According to our present knowledge of the ornithosis system the animal species can be listed in decreasing order of their ability to fix C as follows mammals (rabbit guinea pig man) psittacines pigeon, turkey and chicken Whether duck antibody is similar to turkey or chicken antibody remains to be determined Work is in progress to determine whether ICF antibody represents the DCF antibody and has lost CF ability because of inactivation of reactive sites

In regard to the intradermal test the results of skin testing naturally infected ornithotic turkeys confirm previous observations made on cutaneous hypersensitivity in experimentally infected chickens[11] The good correlation between the serologic and allergic reactor rates suggests the usefulness of the intradermal test for detection of ornithosis exposure From a practical viewpoint skin testing large numbers of turkeys in the field was found to be a less cumbersome procedure than bleeding the birds and then processing the blood samples According to our experience in testing more than 2000 turkeys in the field 5 or 6 birds were skin tested for every bird bled Of all the tests proposed it is felt that the intradermal test is the most practical for extensive ornithosis survey studies

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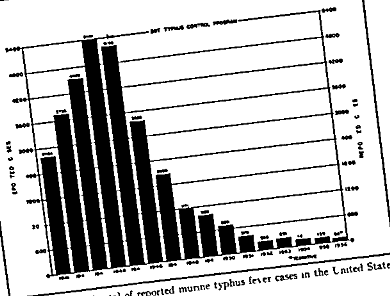


FIGURE 1 Annual total of reported murine typhus fever cases in the United States between 1941 and 1956

The period 1941 to 1944 might be considered the culmination of the classic era of murine typhus in the United States which began in 1913 with Paullin s description This period might be characterized as follows

(1) There was a steady increase in the reported cases of murine typhus from 2784 in 1941 to 5401 in 1944 as shown in FIGURE 1

(2) There was an increasing number of deaths ascribed to murine typhus as shown in TABLE 1 108 in 1941 and 193 in 1944

(3) There were sizable populations of oriental rat fleas in many parts of the southeastern United States Davis (1945) reported that the index of oriental rat fleas varied from as low as 3 to as high as 25 per rat in San Antonio Texas in 1944 Data from Public Health Service surveys of seaport towns made before 1940 and after 1944 before the initiation of DDT dusting suggest that in many parts of the southeastern United States the oriental rat flea index was rather high from 1941 to 1944 For example Cole and Koepke (1947) have published data showing an oriental rat flea index in Savannah Ga varying between 1 and 12 fleas per rat during the years 1932 and 1933 while Ludwig and Nicholson (1947) showed that in May and June of 1945 the index varied from 5 to as high as 57 fleas per rat depending on the type of habitat and date of trapping It is reasonable to assume that in the intervening years 1941 to 1944 the oriental rat flea population in Savannah was at comparable levels

(4) There was a rather high index of murine typhus infections in domestic rats As one example Davis (1948) has written that the blood of 35 per cent of the roof rats and 51 per cent of the Norway rats in San Antonio Texas gave

THE CHANGING PICTURE OF MURINE TYPHUS IN THE UNITED STATES

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The picture of murine typhus first appeared in the United States in 1913 when J E Paullin reported six cases in Atlanta Ga The epidemiological studies of Maxcy (1926 and 1929) in the southeastern part of the country showed conclusively that endemic typhus was a milder disease than epidemic typhus that it occurred in individuals who were not infested with body lice that cases occurred more commonly in summer and fall than in winter and early spring as in the case of the classic louse borne typhus, and that there might be a rodent reservoir of the disease The investigations of Dyer Rumreich and Badger (1931) showed that the oriental rat flea (*Xenopsylla cheopis* Rothschild) was the vector of murine typhus In this same year Mooser Castaneda and Zinsser (1931) established the fact that the domestic rat was the reservoir of this disease In the 1930s the Weil Felix agglutination *test was used increasingly in the laboratory to confirm the clinical diagnosis of* murine typhus The number of cases of murine typhus reported annually increased from 511 in 1930 in four wavelike surges with peaks of 2070 in 1933 2393 in 1937 2996 in 1939 and 5401 in 1944 when the greatest number of cases was reported Bengston (1941) and Bengston and Topping (1941) reported that the complement fixation test was specific for endemic typhus fever This was followed by a later paper of Brigham and Bengston (1945)

In 1944 and 1945 a new approach to the control of murine typhus—the use of DDT to control the oriental rat fleas that transmit the murine typhus rickettsiae from the rodent reservoir to man—was begun in two widely separated areas Savannah Ga where Ludwig and Nicholson (1947) carried out much of the research basic to the cooperative CDC State Health Department murine typhus control program begun in the last half of 1945 and San Antonio Texas where Davis (1945) carried out equally significant experiments on the control of rat fleas with DDT

In 1949 chloramphenicol (Chloromycetin) came into the picture as the first of a new series of antibiotics that were highly effective in controlling the rickettsial diseases (Smadel *et al* 1949) The decline in the number of cases and deaths due to murine typhus coincides with these last three significant discoveries the DDT program to control oriental rat fleas thus breaking the transmission cycle from rodents to man the complement fixation test for laboratory *confirmation of suspected cases and the widespread use of anti*biotics for the treatment of human cases

It is the purpose of this paper to show the changing picture of murine typhus in the United States by comparing two four year periods 1941 to 1944 and 1953 to 1956

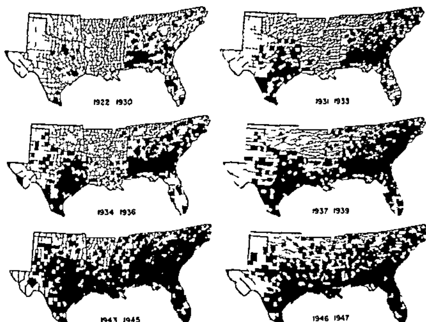


FIGURE 2 Counties in 11 southern states reporting cases of endemic typhus fever between 1922 and 1947

ported from Coffee County in 1943 but in a door to-door survey 211 persons said they had had the disease Blood specimens were obtained from 177 of the 211 reputed cases and 135 revealed positive results in complement fixation and/or Weil Felix tests Therefore more than twice as much typhus occurred in Coffee County in 1943 than was reported Davis and Pollard (1946) made a careful complement fixation survey of 4219 persons in San Antonio Texas between February and June of 1945 They estimated that about 700 persons had been infected each year during the ten years from 1935 to 1945 although the peak number of cases reported by the San Antonio Health Department was 91 in 1944

C L Williams (1949) has summarized the thoughts of many people interested in murine typhus control during this period in his discussion of the typhus control program initiated in 1946 by the Georgia State Health Department and the Communicable Disease Center in Brooks Thomas Grady and Decatur counties in southwestern Georgia He wrote Our workers first made a house to-house survey in these counties to determine the number of cases of murine typhus that had occurred during the one year period immediately preceding the survey This study revealed that about one third the number of cases had been reported by physicians If this figure is true for the whole typhus area it would indicate that there actually occur three times as many cases as are reported in 1944 this would have been about 16 000

TABLE 1

REPORTED DEATHS DUE TO TYPHUS IN THE UNITED STATES 1940 TO 1955

Year	Murine typhus	Unspecified†
1940	84	37
1941	108	39
1942	149	60
1943	154	58
1944	193	80
1945	173	75
1946	128	35
1947	88	31
1948	68	15
1949	2	23
1950	—	13
1951	—	16
1952	—	6
1953	—	6
1954	—	7
1955	—	5

* Source *Vital Statistics of the United States* the years 1940 to 1944 published by the Department of Commerce Bureau of Census the years 1945 through 1949 by the Federal Security Agency Public Health Service National Office of Vital Statistics and the years 1950 through 1955 by the Department of Health Education and Welfare Public Health Service National Office of Vital Statistics Bethesda Md

† Excludes Brill s disease and other rickettsial diseases

a positive complement fixation test for murine typhus in 1944 to 1945 He wrote an analysis of presence of antibodies for typhus shows that [rats in] grain mills had a significantly higher per cent than stores or residences' This localization of typhus in such places as granaries mills and along railroad sidings was noted by many investigators

(5) There were small epidemics of murine typhus in cities and fairly wide spread infections in rural areas in the southeastern United States Meleney (1941) has given a most interesting summary of the cases of endemic typhus occurring in cities such as Savannah Macon and Atlanta Ga for the years 1922 to 1939 including an outbreak of 75 cases in Nashville Tenn in 1939 This trend continued from 1939 up through 1944 for Chatham County (Savannah) and Fulton County (Atlanta) Ga had 139 and 69 cases respectively in 1944 (Communicable Disease Center 1947) Coffee County Ala may have had 500 cases in 1943 according to Hill and Ingraham (1947) Meleney s maps are similar to those shown in FIGURE 2

(6) Typhus may have been under reported in the southeastern United States from 1941 through 1944 There was a general feeling among health authorities that considerably more murine typhus existed than was reported to health departments During the war years it was not possible to conduct an accurate survey to determine the status of typhus because of the shortage of manpower and the emphasis on malaria control However the survey made by Hill and Ingraham (1947) in 1943 in Coffee County Ala may be considered typical of the typhus situation at that time in parts of the Southeast These investigators wrote that only 61 cases of murine typhus had been officially re

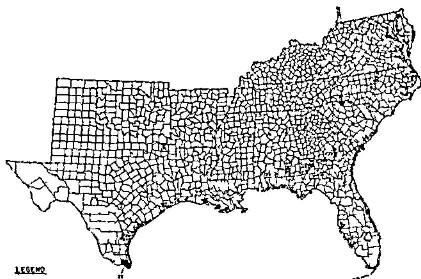


Figure 4 Reported cases of *murine* typhus in the southeastern United States during 1954

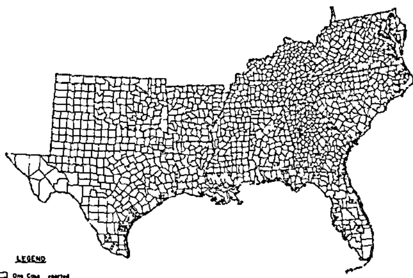


Figure 5 Reported cases of murine typhus in the southeastern United States during 1955

(7) There were no effective drugs for the treatment of clinical cases of murine typhus The authoritative *Textbook of Medicine* edited by Cecil (1940) which was widely used during the years between 1941 and 1944 stated simply There is no specific treatment for typhus Treatment should be directed toward supporting the patient and eliminating as far as possible the source of exhaustion until spontaneous recovery can occur Continuous rest in bed and nursing are most essential'

(8) Control of murine typhus was based on good general sanitation to limit rodent and oriental rat flea populations supplemented by fumigation rat trapping ratproofing of buildings and rat poisoning with relatively ineffective rodenticides such as red squill

From 1953 through 1956 quite a different picture of murine typhus may be seen in the United States as indicated by the following data

(1) There was a steady decrease in the reported cases of murine typhus—from 221 in 1953 to 98 in 1956 (FIGURES 1 3, 4, 5, and 6) This is the first year since 1924 in which fewer than 100 cases of murine typhus have been officially reported to the Public Health Service

(2) There have been no officially recorded deaths due to murine typhus since 1950 although a number of deaths due to unspecified typhus have been registered (TABLE 1)

(3) The population of oriental rat fleas has been highly variable throughout the southeastern United States In a study made in Atlanta Ga in August 1957 a total of 482 oriental fleas was collected from 205 rats an average of slightly more than 2 fleas per rat The data for a series of rat rat flea and

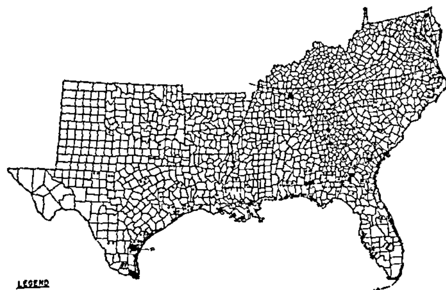


FIGURE 3 Reported cases of murine typhus in the southeastern United States during 1953

TABLE 2
DATA ON RATS TRAPPED RAT SERA TESTED FOR TYPHUS AND NUMBER OF *XENOPSYLLA CHEOPIS* FOUND ON RATS TRAPPED IN FULTON COUNTY GA FROM 1945 TO 1957

Year	Number rats trapped	Number rat sera tested for typhus	Number rat sera positive for typhus	Number rats with *X cheopis*	Number *X cheopis*
1945	150	35	23	87	761
1946	507	416	63	74	433
1947	1288	1243	255	257	1496
1948	657	641	80	35	67
1949	347	329	19	99	396
1950	190	190	7	59	132
1957	205	195	4	74	482

Twenty-one sera anticomplementary

cases were appraised as not typhus In Georgia McCroan *et al* (1955) were able to confirm only 13 of 41 reported cases of typhus in 1953 They wrote One of the confirmed typhus cases was reported as Rocky Mountain spotted fever Five of the 29 unconfirmed cases were classified as presumptive but 24 of these cases remained diagnostic problems even after prolonged study

(7) There are effective drugs for the treatment of murine typhus such as chloramphenicol and the tetracycline agents such as Aureomycin and Terramycin However these new antibiotics have had an important effect on the reporting of rickettsial infections to health departments According to McCroan *et al* (1955) The rickettsial infections are so well masked by antibiotics that many cases are aborted and diagnosis with complete laboratory confirmation is a rarity Development of complement fixation antibodies is delayed sometimes for 6 or more months Confirmation of diagnosis in recent years has been achieved largely as the result of epidemiological follow up of cases by public health agencies

(8) Control of murine typhus today is based on good general sanitation rat trapping ratproofing and rat poisoning with red squill and a number of new rodenticides such as ANTU 1080 and particularly the anticoagulants such as warfarin and pival which give effective rodent control thus limiting the flea populations Since 1944 a variety of new organic insecticides such as DDT dieldrin and heptachlor have been developed These are cheap and effective and they have a long residual action in controlling oriental rat fleas

Between 1926 and 1945 the majority of the cases of murine typhus were reported from the southeastern and the Gulf Coast states In fact the distribution of cases in this area was so pronounced that the Communicable Disease Center manual (1949) included the statement Over 90% of the cases occur south of a line drawn from Charleston South Carolina to Dallas Texas Data on cases of murine typhus reported in this area for the 7 southeastern states of South Carolina Georgia Florida Alabama Mississippi Louisiana and Texas are presented in TABLES 3 and 4 It will be noted that in the years 1941 to 1944 from 90 to 93 per cent of the cases of typhus were reported from these states while in the later period from 1953 to 1956 82 to 86 per

rat blood surveys are summarized in TABLE 2 These figures indicate that, while typhus infection rates in Norway rats have not changed significantly since 1950, the number of oriental rat fleas particularly the average number of fleas per infested rat increased in Atlanta in 1957

(4) There was a rather low index of murine typhus infection in rats in some parts of the southeastern United States and a considerably higher index in other parts In laboratory tests of 195 rat sera collected in Atlanta, Ga during August and September 1957, only 4 sera gave indications of typhus infections by complement fixation and the titers were low in all these specimens in the range of 1 4 to 1 16

(5) There have been no murine typhus epidemics in cities or in rural areas of the magnitude seen in the 1930s or early 1940s Quinby and Schubert (1953) made a special effort to 'detect association of proved cases in time place and persons thus defining areas considered as outbreaks' This was successful only as follows

Brundidge Ala	1950	2 cases
Samson Ala	1951	2 cases
Santa Rosa County Fla	1951	2 cases
Columbia S C	1951	6 cases

(6) Typhus may have been over reported in the southeastern United States from 1953 through 1956 This trend seems to have become apparent beginning in 1948 Quinby and Schubert wrote From 1948 through 1951 351 reported and 99 unreported cases of murine typhus were appraised in 8 southern states After complement fixation testing 58 per cent of the reported cases and 33 of the unreported cases were confirmed At least 25 per cent of the reported

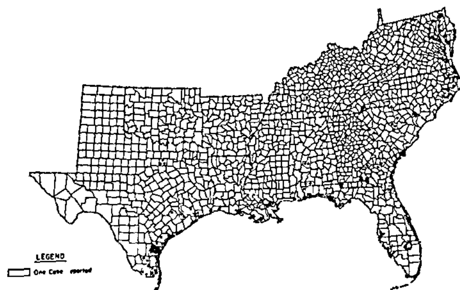


FIGURE 6 Reported cases of murine typhus in the southeastern United States during 1956

ments It is probable that the widespread use of many of the newer rodenticides such as warfarin pival 1080 or ANTU has had an over all influence in reducing the number of older rats with typhus infections thus reducing the reservoir of infected rodents Since 1949 the increasing use of new antibiotics such as chloramphenicol Terramycin and Aureomycin may have affected the reporting of cases by delaying the development of murine typhus antibodies in the blood and making positive laboratory confirmation of this disease difficult or hopeless

It is impossible to predict whether murine typhus may eventually be eradicated from the United States as seems possible with malaria or whether the disease will recur with increasing frequency The Atlanta survey made during the summer of 1957 did indicate that the average rat flea index was high enough (an average of 2 4 oriental rat fleas per rat) for the transmission of murine typhus rickettsiae from the rodent reservoir to man but that the level of typhus infection in rats was very low For this reason very few typhus cases should be expected in Atlanta this year It is worth noting however that the few typhus infections found in rats in Atlanta in 1957 occurred in typical typhus areas namely in low income housing areas in old business districts and around milling companies In some of these areas there were between 30 and 40 oriental rat fleas per rat a dangerously high index if the typhus infection rate should increase in rats in these areas In this connection it is interesting to note that the *Morbidity and Mortality Weekly Report* for August 23 1957 reported 76 cases of murine typhus for the period January 1 to August 17 1957 as compared with 70 for the comparable period in 1956 It is therefore possible that more cases of murine typhus will occur in 1957 than in 1956 and that murine typhus will become a more severe problem than it has been in the last several years

Summary

The changing picture of murine typhus in the United States is discussed by comparing two 4-year periods 1941 to 1944 and 1953 to 1956

In the first period the number of cases of murine typhus showed a steady increase from 2784 reported in 1941 to 5401 in 1944 in the latter period the number of cases of this disease decreased from 221 in 1953 to 98 in 1956 There has also been a decrease in the number of deaths ascribed to murine typhus The number of deaths varied from 108 in 1941 to 193 in 1944 Since 1950 not one death has been officially recorded as due to murine typhus fever although a number of deaths due to un pecified typhus have been registered

In the first period 90 to 93 per cent of all the reported cases in the United States occurred in seven states South Carolina Georgia Florida Alabama Mississippi Louisiana and Texas in the second period 82 to 86 per cent were con entrated in this area

In the first period treatment of murine typhus was largely symptomatic today there are broad spectrum antibiotics such as Chloromycetin Aureomycin and Terramycin which give quick and effective cures

These new drugs however mask all types of rickettsial infection so that diagnosis with laboratory confirmation is difficult or rare The use of these

Table 3

Reported Cases of Murine Typhus in Seven Southeastern States 1941 to 1944

	1941	1942	1943	1944
South Carolina	105	192	193	178
Georgia	946	1154	1256	1182
Florida	196	313	314	484
Alabama	299	380	629	892
Mississippi	66	58	133	178
Louisiana	200	165	232	283
Texas	733	1204	1452	1740
Subtotal	2545	3466	4209	4937
U S total	2784	3722	4528	5401
Percentage in 7 southeastern states	90%	93%	93%	90%

cent of the cases were reported from this area In 1956 51 cases or more than one half of the total for the entire United States were reported from Texas 17 of them were confirmed by laboratory diagnosis

Many people have speculated on the reasons for this decline in murine typhus since 1944 A number of public health officials feel that the extensive DDT dusting program carried out by state and local health departments in cooperation with the Communicable Disease Center played an important part in the initial lowering of the typhus rate from 1944 to 1950 There was a considerably larger reduction in the number of cases of typhus in urban than in rural areas Undoubtedly the re emphasis on basic sanitation has played some part in reducing rat and rat flea populations in cities since the end of World War II particularly programs for new municipal housing slum clearance ratproofing of business and food handling establishments better garbage collections and sanitary landfills In addition there has been an ever increasing reliance by private industry on exterminators for the control of rodents and all types of insects including fleas in business and food handling establish

Table 4

Reported Cases of Murine Typhus in Seven Southeastern States 1953 to 1956

	1953	1954	1955	1956
South Carolina	11	11	6	7
Georgia	41	32	15	9
Florida	10	5	11	6
Alabama	19	23	20	10
Mississippi	3	—	3	0
Louisiana	7	3	7	2
Texas	92	64	52	51
Subtotal	183	138	114	85
U S total	221	163	135	98
Percentage in 7 southeastern states	82%	84%	84%	86%

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new drugs has therefore probably obscured the true incidence of this disease today

In the first period the prevention of murine typhus was based on controlling infected rat fleas by fumigation rat trapping rat poisoning with relatively ineffective rodenticides such as red squill ratproofing and sanitation Today in addition to these methods there are powerful insecticides such as DDT dieldrin and heptachlor which give excellent control of infected rat fleas for weeks or months Many new rodenticides have been developed such as ANTU 1080 and particularly the anticoagulants such as warfarin pival and fumarin which give effective rodent control and thus limit the flea population

Finally one can speculate that improved sanitation programs in cities throughout the United States and the increasing reliance of private industry on commercial pest control services to control rodents and all types of insects have had a long term influence on the steady and continued decrease of murine typhus in the United States

Acknowledgments

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AIR-BORNE TRANSMISSION OF Q FEVER: THE ROLE OF PARTURITION IN THE GENERATION OF INFECTIVE AEROSOLS*

By Hartwell H. Welsh, Edwin H. Lennette, Francis R. Abinanti,† and John F. Winn‡

Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley, Calif.

Prior to 1944–1945, Q fever as a disease of humans was a clinical rarity seen as a naturally occurring illness only in Australia. In the past thirteen years the disease has shown itself on all the continents and the British Isles in circumstances varying from sharply defined and explosive outbreaks to smoldering and continuous endemics. It now appears that it can be found in any part of the world where an effort is made to detect it. Whether the disease has spread from Australia to the rest of the world in the years since its original description by Derrick[1] in 1937 or whether propitious circumstances led to its recognition first in Australia is a question that cannot be answered at present. In any case Q fever has become a disease of world-wide importance and the considerable effort expended in attempts to define the natural history of this disease has been highly productive, especially during the last decade.

Derrick[2] and Stoker and Marmion[3] have presented very fine reviews of the findings relative to the epidemiology of the disease in general; beyond a brief synopsis of this subject the present paper will deal rather with data concerning the actual generation and persistence of infective sources of the agent.

The Epidemiology of Q Fever

Sources of the agent in nature. *Coxiella burnetii* has been isolated from at least 22 species of ticks,[4-31] 1 insect,[32] 8 mammals[33-49] and at least 1 bird.[50] Many of the ticks found naturally infected have been shown capable, under laboratory conditions, of transmitting the infection by bite. In some species stage-to-stage transfer of the agent occurs and, in others, the agent is transmitted transovarially. The tissues and excreta of such infected ticks have been shown to be potential sources of large numbers of the organism.

Among domestic animals, cattle, sheep, and goats would appear to be the most important sources of the organism because of their world-wide distribution and large numbers. *C. burnetii* has been isolated from the tissues of cattle and sheep and from the secretions and excretions of all three species. The placenta is an especially rich source of the agent. In the case of sheep it has been shown that the wool itself[1,5] may be heavily contaminated with the organism. This is presumably true of cattle and goat hides as well, although this has not as yet been firmly established.

* The work on which these observations are based was supported by Grant E-195 from the National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md.

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Other domestic animals and wild or domestic birds or fowl are perhaps potential sources of *C burnetii* but are less frequently involved in the disease cycle than are the three major domestic animal species

Man has on occasion been the source of rickettsiae[53, 54] in tissues from fatal cases and from sputum and urine during the acute illness Person to-person transmission of the agent has been of importance only in isolated instances[55] and does not seem to be of consequence in spread of the disease

Modes of transmission to man or to domestic animals Transmission of the infective agent from natural sources to man or to domestic animals may occur in a number of ways depending upon the situation In the case of man the association between infection and exposure to domestic animal or their products is very striking exposure may be occupational or residential in type and intense or merely casual in nature In the case of domestic animals a similar association may exist but it is considerably more difficult to establish because in most instances the occasions on which domestic animals can be kept from close association with other livestock are far less common

Definite epidemiological evidence exists that *C burnetii* may be carried over considerable distances on inanimate objects such as clothing[56] wool[57] hair[58] straw[59] packing materials[60] and dust[61] More direct evidence exists in the case of wool

As mentioned previously the possibility of transmission by the bite of infected ticks is a potential method utilized by the agent for transfer from one host to another However the evidence that such a method is of any real consequence is scant On the other hand the tissues and excreta of infected ticks have undoubtedly been responsible for some human infections brought about by handling of items thus contaminated

In the same manner that is through manipulation or agitation of contaminated surfaces the ground and soil of areas housing infected livestock may be through the generation of dusts containing infectious particles a very important method for transmission of the agent to susceptible hosts[62, 63]

Routes of infection for man and animals Several portals of entry into the susceptible host exist in the case of *C burnetii* Thus laboratory animals may be infected through the unbroken (probably abraded) skin by subcutaneous intradermal or intraperitoneal inoculation through ingestion of infective materials by placement of infective materials on the conjunctiva by intranasal instillation or through inhalation of artificially generated infective aerosols It is assumed that the same potential routes of infection exist in the case of larger animals and man as a matter of fact some of these routes have been shown epidemiologically to be of importance in producing infection under natural conditions

With respect to man at least a general survey of the reported epidemiological findings indicates that the majority of cases of Q fever may well have resulted from contact with air borne rickettsiae taken into the body either through inhalation or through deposition onto the ocular conjunctivae or other mucous membranes Of these possibilities the respiratory route constitutes the most probable means of ingress for the agent

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Infective aerosols may be classified in several ways and for present purposes they may be classified as either primary or secondary in type. A primary aerosol may be defined as one generated directly by or in direct association with an infected animal, for example, an infective aerosol arising during parturition of an infected animal. A secondary aerosol then is one produced by some factor or action or incident temporally or spatially removed from those giving rise to primary aerosols. Secondary aerosols are produced during the handling or processing of contaminated materials such as wool and hides at some distance in time and/or space from, for example, the site of the original parturition.

Infection with *C. burnetii* can obviously be produced by both primary and secondary aerosols. The production of secondary aerosols is relatively easy; indeed, many instances of infection recorded in the literature appear to have been unavoidable. The handling of contaminated materials in plants or places far removed from the actual animal sources responsible for the contamination has on occasion produced outbreaks of Q fever in wool plants,[57] laundries,[56] and in such unlikely places as an art school[60] and a locksmith shop.[59] Such outbreaks appear to be possible whenever objects contaminated by infected animals are handled. In the immediate environs of infected live stock, such infective aerosols are readily produced by a mere agitation of the ground surface.[6, 63]

Secondary aerosols are highly important epidemiologically, being a major factor in the dissemination of the disease to new areas and populations and perhaps they play a greater role in the production of human illness than do the primary aerosols; nevertheless the circumstances surrounding the production and persistence of primary aerosols are of more than passing interest. It is the circumstances surrounding the production of these primary aerosols with which we shall be concerned in the remainder of this report.

The Production of Aerosols of *C. burnetii* by Infected Animals

Many prior observations cited above had shown that a large percentage of human illness due to Q fever occurred in individuals exposed to domestic livestock either by occupation or residence. In attempts to clarify this relationship, we found that the rickettsia is excreted by infected animals during parturition in the placenta and birth fluids and afterwards in the feces. Furthermore, it appeared likely that human and perhaps also animal infection could be acquired by exposure to aerosolized infectious materials. Consequently, air sampling studies were undertaken to determine the factors related to the appearance and persistence, if any, of *C. burnetii* in the air of the environs of known infected animals. A group of pregnant sheep was inoculated intravenously with varying doses of *C. burnetii*. Six animals were used in the studies, and the air sampling trials, using a cotton plug sampler, were conducted over a period encompassing the ante partum, parturient, and post partum states of each infected sheep.

Methods and Materials

Inoculation of animals Six sheep in 2 groups of 3 animals each were inoculated intravenously via the external jugular vein Each animal of the first group received 1 × 10⁹ hamster infective doses (intraperitoneal) and each animal of the second group received 1 × 10⁴ hamster infective doses of *C burnetii* The inoculum consisted of 10 ml of infected yolk sac diluted to the appropriate concentration with sterile skimmed milk

Housing of infected animals The sheep used in these studies were housed in isolation cubicles of the following dimensions width 3 feet length 8 feet and height 8 feet total volume 192 cubic feet Each cubicle was completely enclosed by wooden tongue and groove walls and ceilings The floors were of cement A solid plywood door at one end of the cubicle opened onto a common interior alleyway The doorstep was raised 8 inches from the concrete floor At the other end of the cubicle a door half glass gave access to an outer alleyway open to the exterior but covered by a roof

Ventilation in each cubicle was accomplished by natural air flow through a 6 × 18 inch screened opening near the bottom of the door to the outer alley way There were no other gross openings in the cubicles Traffic between the cubicles was confined to the inner alleyway the usual isolation tech niques were carried out at all times However the possibility that traffic or minute openings between the cubicles could result in cross contamination cannot be ruled out

The sheep were placed in these pens prior to inoculation and were kept there throughout the entire study

Once each week up to the time of parturition the pens were cleaned and fresh wood shavings added to a depth of approximately 3 inches Pens were not cleaned for 2 weeks following parturition Additional shavings were added as necessary however to provide a clean dry surface for the comfort of the animals Fresh water and feed were provided daily

Types of air sampling equipment and preparation for use Cotton plug samplers of the type described by Rosebury[64] and as modified by S Division Camp Detrick Md were used throughout these studies

Clean glass sampler shells were sterilized in an autoclave at 18 lb pressure for 30 min and then packed with wafers punched from a single roll thickness of sterile No 1 absorbent cotton The wafers were cut with a three fourths inch diameter leather punch and were thus similar with respect to thickness diameter and fiber length The wafers were forced into the glass shell against a stainless steel retaining screen previously placed in position Sufficient cotton wafers were placed in each sampler shell to provide resistance to air flow equal to displacement of 28 to 32 mm of mercury at a flow rate of 20 l min

After packing and calibration the samplers were placed in cotton stoppered glass tubes and sterilized by dry heat (350 F for 60 min) Tests indicated that the resistance and the general physical characteristics of the samplers were not affected by this procedure The samplers were kept in closed con tainers until used in the sampling trials

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The Production of Aerosols of *C. burnetii* by Infected Animals

Many prior observations cited above had shown that a large percentage of human illness due to Q fever occurred in individuals exposed to domestic livestock either by occupation or residence. In attempts to clarify this relationship we found that the rickettsia is excreted by infected animals during parturition in the placenta and birth fluids and afterwards in the feces. Furthermore it appeared likely that human and perhaps also animal infection could be acquired by exposure to aerosolized infectious materials. Consequently air sampling studies were undertaken to determine the factors related to the appearance and persistence of any of *C. burnetii* in the air of the environs of known infected animals. A group of pregnant sheep was inoculated intravenously with varying doses of *C. burnetii*. Six animals were used in the studies and the air sampling trials using a cotton plug sampler were conducted over a period encompassing the ante partum, parturient and post partum states of each infected sheep.

TABLE 1
RESULTS OBTAINED FROM AIR SAMPLING CARRIED OUT IN PENS HOUSING PREGNANT SHEEP ARTIFICIALLY INFECTED WITH *COXIELLA BURNETII*. SAMPLING DONE PRIOR TO PARTURITION

	Pen 9 Sheep 12		Pen 10 Sheep 13		Pen 11 Sheep 2		Pen 12 Sheep 7		Pen 13 Sheep 4		Pen 14 Sheep 38	
Dosage	1 × 10		1 × 10		1 × 10		1×10^4		1×10^4		1×10^4	
Placenta	Out*		In†		Out*		In†		Out*		In†	
Date	P	CS	P	CS	P	CS	P	CS	P	CS	P	CS
January 16	−14	−									−20	−
17	−13	−									−19	−
18	−12	−									−18	−
19	−11	NC									−17	NC
20	−10	NC									−16	NC
21	−9	−									−15	−
22	−8	−									−14	−
23	−7	−									−13	−
24	−6	−									−12	−
25	−5	NC									−11	NC

* Placenta removed from pen immediately postpartum
† Placenta left in pen throughout entire sampling period
P = Days before (−) or days after (+) parturition
C S = Results of hamster inoculation of the cotton plug air sample taken on a given day
\+ = *C. burnetii* present in air sample
− = *C. burnetii* not present in sample
NC = No sample collected

On January 28, parturition occurred in sheep No. 13 in pen 10, and it will be noted from TABLE 2 that the rickettsiae were recovered from the air of this pen on this day and that they were present therein for at least 14 days thereafter. The air sample taken in this same pen on the day before parturition did not contain the rickettsiae. It will also be observed that on the day prior to parturition in sheep No. 12, pen 9, rickettsiae were present in the air of the pen and presumably represented contamination from the adjacent pen 10. It is possible that contamination of the atmosphere in the other pens may have occurred, but since sampling in pens 11 to 13 was not done, no statement can be made. However, no rickettsiae were recovered from the air of pen 14, which was the farthest removed from pens 9 and 10. Parturition in sheep No. 12 on January 30 was also associated with the presence of the rickettsiae in the air of the pen (pen 9) in which it lambed, and the organisms were present continuously for at least 11 days after the parturition.

Essentially the same situation was observed in the cases of sheep Nos. 2, 4, 7, and 38, that is, parturition was accompanied by the presence of rickettsiae in the air during an appreciable period of time. Some cross contamination presumably occurred between the pens in several instances, since the rickettsiae could occasionally be detected in pens in which the animals had not yet lambed. This sporadic appearance in these pens is in contrast to the essentially continuous presence of the organism in the same pen after the occupant had lambed. These observations on the appearance of rickettsiae in the air

After use in the trials the cotton plug portion of the sampler was removed aseptically from the glass shell placed in a sterile screw capped jar and stored at −20° C until it was prepared for testing in laboratory animals Prior to testing the jar containing the cotton plug was placed in cold running tap water until the sampler was completely thawed The cotton plug was then broken up with the use of sterile forceps and to each sample was added 25 ml of a 0 4 per cent solution of Triton X 100* in distilled water The mixture was agitated for 20 min and 5 ml of the resulting supernatant fluid was pipetted off *Penicillin was added to a final concentration of 400 units/ml and the mixture allowed to incubate for 4 hours at 4 C* prior to inoculation into animals

Inoculation of laboratory animals Hamsters six to eight weeks of age were used as test animals in these studies Each mixture was inoculated into four hamsters each animal receiving 1 ml intraperitoneally Six weeks after inoculation the animals were bled by cardiac puncture and their sera were examined for the presence of antibodies to *C burnetii* by the complement fixation technique The presence of antibody in the serum of one or more animals of a test group of four was regarded as evidence of infection and as an indication that the specimen inoculated had contained *C burnetii* (large scale studies had indicated that the hamster population in use had had no previous experience with the organism) During the six week test period uninoculated control animals were left in the same quarters as the inoculated hamsters Complement fixation tests done on sera drawn from these animals after this period were negative indicating that the antibody present in the sera of inoculated test animals was not elicited by cross contaminations

Conditions of air sampling Samplers were placed 4 feet above the surface level of the sheep pens through a small port cut into the interior door The sampling rate was set at 10 l /min by means of calibrated limiting orifices et into the sampling lines Samples were taken for periods of one half to one hour daily starting with a date approximately 1 week prior to the expected parturition in each pen and continuing to a date 2 weeks postpartum in each case

Results

The results of the air sampling trials together with certain ancillary data are presented in TABLES 1 and 2 Air sampling trials were conducted prior to the first parturition in this group of 6 animals sampling being done in pens 9 and 14 The original intention was to determine for each pen whether the ante partum atmosphere was free of the organism The long period of sampling shown for pens 9 and 14 was unintentional and it arose because parturition was not as imminent as was believed at the time the sampling was initiated However the prolonged observations made on these 2 pens show quite well that no rickettsiae at least in detectable amounts were present in the air during the period between inoculation of the animals and the occurrence of the first parturition in the group a period of 12 days

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TABLE 3
INFECTIVITY OF PLACENTA BIRTH FLUIDS AND FECES OF PARTURIENT SHEEP INFECTED WITH *COXIELLA BURNETII*

Sheep No	Infectivity titer*			Feces consistently infectious up to postpartum day	Titer of feces on day indicated
	Placenta	Birth fluid	Feces†		
12 (Pen 9)	1×10	NC	1×10^{4} (12 hr)	14	1×10^{2} (14)
13 (Pen 10)	1×10^{2}	1×10^{3}	1×10^{5} (10 min)	15	1×10^{2} (14)
2 (Pen 11)	1×10	1×10^{3}	1×10 (1 hr)	18	1×10^{3} (14)
7 (Pen 12)	1×10^{3}	1×10^{3}	1×10 (10 min)	11	Negative (14)
4 (Pen 13)	1×10	NC	1×10 (4 hr)	14	1×10^{1} (10)
38 (Pen 14)	1×10	NC	1×10^{7} (4 hr)	14	1×10 (14)

Infectivity titers of material expressed as 50 per cent hamster infective doses per gm or ml

† First postpartum specimen collected 10 min to 12 hr postpartum as indicated

the stools is open to conjecture It is possible that the presence of the organism in the stool represents true excretion in the sense that the organisms were released from some focus in the body and were excreted into the intestinal tract or that multiplication may have occurred in the intestinal mucosa On the other hand the organisms in the stool may represent mere passive contamination due to the ingestion of contaminated food or inhalation of infectious materials subsequently swallowed Experimental work directed to a firmer understanding of these points is highly desirable

That the act of parturition itself is responsible for the initial contamination of the air is well illustrated in TABLE 4 which gives the chronological sequence of events at parturition as they relate to the first appearance of the rickettsiae in the air In the case of sheep No 38 the last sample of air taken before parturition was obtained approximately 18 hours before the birth of the lamb No rickettsiae were detectable in this specimen However a sample taken at an interval of about 3 to 5 hours after the parturition did contain demonstrable numbers of the organism In the case of sheep No 2 rickettsiae were detected in air samples taken 1 to 2 hours after parturition The air of this same pen had not contained the rickettsiae 24 hours prior to parturition The most interesting findings pertain to sheep No 13 As can be seen from TABLE 4 air sampling was being conducted for 55 minutes before and for 5 minutes after the first lamb of a set of twins was born The specimen of air collected during this 1 hour period was negative for rickettsiae even though sampling was carried out for about 5 minutes after the birth Air sampling was resumed using fresh filters at 2 54 P M and continued for 10 minutes being discontinued just as the second lamb was born This air sample contained rickettsiae indicating that the organisms appeared in the air within 5 to 24 minutes after the birth of the first lamb (worthy of remark is the fact that the air samplers were in the normal position as described earlier that is no attempt was made to place the samplers in especially favorable locations near the animal during the actual parturition) Offhand it is difficult to see how more direct evidence implicating parturition in environmental

TABLE 2
RELATIONSHIP BETWEEN PARTURITION OF SHEEP INFECTED WITH *COXIELLA BURNETII* AND APPEARANCE OF THE RICKETTSIA IN THE AERIAL ENVIRONMENT

	Pen 9 Sheep 12		Pen 10 Sheep 13		Pen 11 Sheep 2		Pen 12 Sheep 7		Pen 13 Sheep 4		Pen 14 Sheep 38	
Dosage Placenta	1 × 10 Out		1 × 10 In		1 × 10 Out		1 × 10⁴ In		1 × 10⁴ Out		1 × 10 In	
Date	P	CS	P	CS	P	CS	P	CS	P	CS	P	CS
January 26	−4	−									−10	−
27	−3	−	−1	−							−9	NC
28	−2	−	0	+							−8	−
29	−1	+	+1	+							−7	−
30	0	+	+2	+							−6	+
31	+1	+	+3	+							−5	NC
February 1	+2	+	+4	+							−4	+
2	+3	+	+5	+	−1	−			0	+	−3	NC
3	+4	NC	+6	NC	0	+			+1	+	−2	+
4	+5	+	+7	+	+1	+	−4	−	+2	+	−1	−
5	+6	+	+8	+	+2	+	−3	+	+3	+	0	−
6	+7	+	+9	−	+3	+	−2	−	+4	+	+1	+
7	+8	+	+10	+	+4	−	−1	−	+5	−	+2	+
8	+9	+	+11	+	+5	+	0	+	+6	+	+3	−
9	+10	+	+12	+	+6	+	+1	+	+7	+	+4	+
10	+11	+	+13	+	+7	+	+2	+	+8	+	+5	+
11	+12	−	+14	+	+8	+	+3	+	+9	+	+6	+
12	+13	+			+9	+	+4	+	+10	−	+7	+
13	+14	−			+10	−	+5	+	+11	+	+8	+
14					+11	−	+6	+	+12	+	+9	+
15					+12	−	+7	−	+13	−	+10	+
16					+13	−	+8	+	+14	−	+11	−
17					+14	−	+9	+			+12	+
18							+10	−			+13	+
19							+11	−			+14	−
20							+12	−				
21							+13	−				
22							+14	−				

Refer to TABLE 1 for explanation of symbols

of pens housing preparturient animals coincidentally with and following parturition in infected animals in adjacent pens agree well with other studies done in this laboratory

From TABLE 3 it will be noted that both the placenta and the birth fluids contained rickettsiae and that the rickettsial content of the former was tremendous It might be assumed therefore that the initial and most important source of rickettsiae was the placenta and to a lesser extent the birth fluids Nevertheless it would appear that the long continued excretion of the organism in the *stool* postpartum also must have contributed to the contamination of the environment On the whole the rickettsial content of the stools was considerably less than that of the placenta The true origin of the rickettsiae in

which the organism could be detected in the air It should be kept in mind however that even though the placenta was removed there still remained the possibility that an appreciable amount of contamination could have arisen from spillage of the infected birth fluids In view of the relatively low rickettsial content of these fluids their ability to give rise to long continuous contamination of the air can be questioned The possible role of infected feces in providing a constantly renewed source of the organism cannot be overlooked The rickettsial content of the feces was in some instances quite high but here the question of cause and effect must be considered that is whether the infected stools were the result of true excretion or of only passive contamination having as its source the infected environment itself

The relative importance of these various materials in determining the time span of aerial contamination with *C burnetii* will be shown only when it is determined whether replenishment of this nature is necessary It is entirely possible that some proportion of the total concentration of rickettsiae initially introduced into the air at parturition will remain airborne in detectable amounts for a considerable period especially under interior conditions

In attempting to elucidate some of the problems concerning the original sources of *C burnetii* in the environment of infected animals we have raised other questions with respect to the persistence of the rickettsiae in such environments The observations made thus far have shown that the rickettsiae are not normally present in the aerial environment of artificially infected pregnant sheep until parturition occurs It is also clear that the act of parturition itself is responsible in some manner for the generation of the primary aerosols of this organism but that the persistence of the agent in the air over periods up to 14 days postpartum may depend on other factors

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TABLE 4
RELATIONSHIP BETWEEN PARTURITION OF ARTIFICIALLY INFECTED SHEEP AND CONTAMINATION OF THE ENVIRONMENT WITH *COXIELLA BURNETII* AS SHOWN BY AIR SAMPLING TRIALS

Sheep No	Date	Sampling Period		Sample results	Remark
		Start	Stop		
38 (Pen 14)	February 4	12 20 P M	1 20 P M	−	17–19 hr prepartal
	5	10 30 A M	11 30 A M	+	3–5 hr postpartum
	6	11 50 A M	12 50 P M	+	30–32 hr postpartum
2 (Pen 11)	2	11 20 A M	11 50 A M	−	24 hr prepartal
	3	12 00 M	1 00 P M	+	1–2 hr postpartum
	4	2 50 P M	3 50 P M	+	28 hr postpartum
13 (Pen 10)	January 28	1 45 P M	2 45 P M	−	(Bag of waters protruding broke as first lamb born at 2 40 P M)
		2 54 P M	3 04 P M	+	(Labor—second lamb born 3 04 P M)
		4 30 P M	5 30 P M	+	1½ to 2½ hr postpartum

contamination could be obtained With regard to the environment of the other 3 animals involved it will be recalled from TABLE 2 that parturition of the animals in the respective pens resulted in either initial or renewed evidence of aerial contamination although the time sequence could not be as closely followed

In discussing TABLE 2 it was seen that the rickettsiae persisted in the air of the pens for several days after parturition The concentration of the inoculum originally administered to the animals apparently had no influence on the length of persistence The air of pens 9 10 and 11 holding those sheep that received 1×10^9 hamster infective doses contained rickettsiae in detectable amounts for 13 14 and 9 days respectively or an average of 12 days postpartum Pens 12 13 and 14 housing the animals that received 1×10^4 doses contained rickettsiae for 9 12 and 13 days for an average persistence in these cases of 11 33 days postpartum

Because of the tremendous concentrations of rickettsiae present in the placental tissues it seemed that removal of this highly infected material might affect the extent to which rickettsiae would continue to be found in the air However in this small series no obvious differences were noted regardless of whether the placenta was removed from the pen or remained there during the entire sampling period

DISCUSSION

In these studies it has been possible to show rather conclusively that the act of parturition is responsible for the generation of primary aerosols of *C burnetii* The actual mechanisms involved are admittedly obscure

In this present series the removal of the highly infective placenta from the pen immediately postpartum had no effect on the length of time during

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HUMAN BRUCELLOSIS WITH SPECIAL REFERENCE TO THE DISEASE IN THE UNITED STATES

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To evaluate the problems encountered in brucellosis it is necessary to understand the nature of the disease and some of the characteristics of the causative organism.

Brucella melitensis, *Brucella abortus* and *Brucella suis* are closely related organisms that cause similar diseases in animals and man. However, there are differences that are reflected in the epidemiology, the reported incidence, the laboratory diagnosis, the clinical aspects, and the therapy of brucellosis. In actuality we are dealing with three diseases. A few of the characteristics of *Brucella* that bear on the problems at hand will be considered.

Br. melitensis and *Br. suis* are less exacting in their growth requirements than is *Br. abortus*. The former grow better on the older laboratory media, require no added carbon dioxide, and can be isolated much more readily from man. Prior to the development of adequate media and techniques *Br. abortus* was seldom isolated from human cases of the disease. Even now the average laboratory routine seldom succeeds in isolating this species. Since the majority of cases of brucellosis in the United States have been due to *Br. abortus*, these facts have influenced the reported vital statistics of the disease.

Another characteristic of *Brucella* that has been of great significance is the tendency for most strains of the organism to undergo dissociation when grown on laboratory media. Until recent years most of the experimental work was done with dissociated cultures. With progressive dissociation there is first loss in virulence and then change in antigenicity. Antigen for the agglutination test made from dissociated cultures does not perform satisfactorily. Surveys[1, 2] in which the same sera were examined in different laboratories have shown great discrepancies in test results even to the degree that sera containing agglutinins in high titer were reported as negative in some laboratories. Unsatisfactory antigens and nonstandard techniques are still too widely in use.

The difficulty in isolating the organism from the patient, together with the lack of dependability and uniformity in the results of the agglutination test, not only has led (and still leads) to inaccuracy of diagnosis but also to a distorted concept of the disease. Since they were forced to rely mainly on a clinical impression, many physicians ceased to employ laboratory results or disregarded them and made diagnoses of brucellosis in the absence of any objective laboratory evidence of the disease. This is not a problem confined to the past but one that is currently still of considerable magnitude. Its bearing on the reported incidence of the disease and on concepts of therapy should be apparent.

A consideration of the nature of the infection is of great help in understanding the problems in diagnosis and therapy of the disease. Brucellosis in both domestic animals and man probably has existed since the dawn of history. The organism has become well adapted to life in these hosts and accordingly

it usually produces a subacute or chronic disease Man tolerates the infection less well than do domestic animals and acute disease may result However this is the exception rather than the rule In exposed populations a few individuals develop serious acute and chronic disease, the rest have subclinical infections or brief or low grade illnesses that escape diagnosis In fact *Brucella* has been cultured from the blood streams of persons with no symptoms of illness, who subsequently remained well Overt disease may range from a brief illness of a few days to a severe febrile illness lasting for weeks or months with one or more relapses The onset can be abrupt or insidious Long term chronic illness may follow either type of onset In acute cases there is a septicemia or an intermittent bacteremia The organisms localize in the tissues particularly in the reticuloendothelial system and viscera and have been recovered from local lesions in practically every tissue of the body In the tissues the organisms are found predominantly intracellularly which gives them protection from the action of antibodies and most drugs Pathologically there is nonspecific hyperplasia of the reticuloendothelial tissue and focal necrosis microabscesses and granulomas throughout the viscera The granulomas are usually minute and consist primarily of collections of epithelioid cells and lymphocytes However large abscesses and extensive granulomatous inflammation may occur The formation of granulomas probably operates further to protect the organism from the defense mechanisms of the body and from administered drugs In persons recovering from acute brucellosis or in those who never have had an acute phase localized lesions may persist for years and produce chronic or recurrent ill health

With localization numerous complications may occur Perhaps the most common is osteomyelitis[2,5] The author has seen cases with this complication caused by all three species of *Brucella* *Brucella* spondylitis with destruction of an intervertebral disc and adjacent vertebral bodies is severely painful and incapacitating Osteomyelitis of any bone may occur the spine pelvis ribs and long bones are the most frequent sites Suppurative arthritis usually monarticular may occur in any joint usually the hip or knee This may arise during the course of systemic infection or may be the presenting complaint without other evidence of illness Other complications are orchitis epididymitis subacute endocarditis cholecystitis chronic pyelonephritis hepatic pericholecystic and subdiaphragmatic abscesses and localized abscesses in any part of the body acute or chronic meningoencephalitis peripheral neuropathies with the auditory and optic nerves most commonly attacked granulomatous lesions of the eye and a severe hepatitis leading to cirrhosis of the liver[6] With local infection in the brain organic brain damage results leaving a variety of sequelae Evidence has been presented that long term chronic infection may be accompanied by organic brain disease and deterioration[7,8] Finally neuropsychiatric disorders occur so commonly in brucellosis that they must be regarded as part of the manifestations of the disease

The disease then presents many aspects ranging from mild or severe acute illness to recurrent or chronic disease persisting for as long as twenty five years The multiple manifestations may require the practice of all the medical specialties in diagnosis and treatment

Incidence

The incidence of brucellosis in man is difficult to determine One index is the number of cases reported through official channels of the various state departments of health In interpreting such data it must be borne in mind that cases are notified on the basis of a physician s diagnosis How truly a reported incidence may represent the real incidence of any disease is dependent upon the criteria used for diagnosis as well as upon the completeness of reporting With brucellosis more than most diseases there has been great variation in the criteria used for diagnosis from rigid proof by isolation of the organism from the patient to clinical diagnosis unsupported by laboratory tests It is obvious that the reported figures by themselves without knowledge of the criteria used are most difficult to interpret

In the two decades following the recognition that *Br abortus* and *Br suis* cause disease in man laboratory techniques were not sufficiently dependable to provide cultural proof of the diagnosis and nonstandard and undependable antigens for the agglutination test were in use as already mentioned Of necessity the physician was forced to rely mainly on his clinical judgment with the result that nearly any vague illness came to be called brucellosis In later years diagnostic criteria used by conservative physicians have become more rigid but even today many physicians arrive at a clinical diagnosis of brucellosis in spite of entirely negative laboratory tests

Difficulties in diagnosis failure to suspect brucellosis and the occurrence of mild cases that go unrecognized all add to the unreliability of the reported statistics of the disease

Nevertheless if one excepts salmonellosis deriving from animal sources brucellosis in man in the United States is still of higher reported incidence than any other disease of animals transmitted to man FIGURE 1 presents the annual incidence derived from notified cases for the period 1927 to 1956[9] There was a steady increase in the number of notified cases up to 1947 reaching a peak of 6321 Since that date the notified incidence has decreased sharply becoming currently a little more than 1000 cases per year Since there was no really significant increase in brucellosis of domestic animals prior to 1947 it is presumed that the increasing number of reported cases during this period was due to a growing awareness of the disease by physicians rather than to a real increase in incidence In recent years other factors have become important (1) the more widespread practice of pasteurization of milk (2) the introduction and indiscriminate use of the broad spectrum antibiotics and (3) the intensification of the control and eradication program for bovine brucellosis Disease due to milk borne brucellosis is now practically limited to the rural areas Some drop in incidence may be due to this factor The broad-spectrum antibiotics are effective in suppressing clinical brucellosis The indiscriminate use of these antibiotics has probably obscured the diagnosis of brucellosis in many instances Such cases do not appear in our vital statistics The drop in incidence of the disease in recent years may be more apparent than real

In the last ten years great strides have been made in the control and eradication of bovine brucellosis Programs in some states have been more active

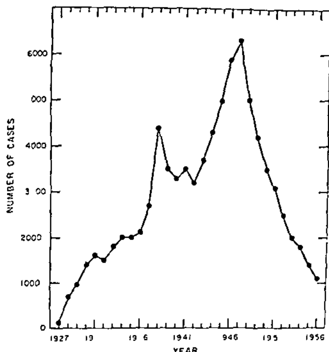


FIGURE 1 Notified cases of brucellosis in the United States from 1927 to 1956 (84 754 cases)

and successful than in others It might be expected that a corresponding relative decrease in the reported incidence of brucellosis in man would result in those states that have achieved the greatest decrease in incidence of bovine brucellosis The annual incidence of brucellosis in man by region and by state for the years 1947 to 1956 is given in TABLE 1

In 1947 brucellosis was reported from every state Seventeen states reported more than 100 cases each and accounted for 78 5 per cent of all cases These same 17 states in 1956 accounted for 67 7 per cent of all reported cases In 1947 over one half of the total reported cases 3332 in number or 52 7 per cent were from the following 7 states Iowa Illinois Texas Wisconsin Minnesota Michigan and California In 1956 52 2 per cent of the total cases were from *the following 7 states Iowa Illinois Minnesota Michigan Kansas Georgia* and Missouri In 4 states there was an absolute increase in reported cases Kentucky (5 to 22) New Mexico (3 to 4) North Dakota (3 to 23) and Wyoming (1 to 4)

The total number of reported cases for 1956 was 1080 This is 17 1 per cent of the total number reported for the peak year 1947 (6321 cases) The percentage of the total contributed by individual states showed an increase in 20 states and a decrease in 26 others

TABLE 2 presents a comparison of the incidence and distribution of notified cases in 1947 and 1956 assuming a uniform rate of reduction for the country

TABLE 1
ANNUAL INCIDENCE OF NOTIFIED CASES OF BRUCELLOSIS IN THE UNITED STATES FOR THE YEAR 1947 TO 1956

	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1947 to 1956
New England	464	203	132	91	86	76	68	48	22	40	1 230
Maine	32	12	12	12	4	8	4	1	2	1	88
New Hampshire	24	8	3	3	1	0	0	1	1	1	42
Vermont	136	36	3	7	45	32	20	13	4	14	310
Massachusetts	76	39	31	22	10	18	15	13	9	15	248
Rhode Island	18	10	5	4	4	3	2	3	1	4	54
Connecticut	178	98	78	43	22	15	27	17	5	5	488
Middle Atlantic	420	392	296	263	180	135	76	66	42	24	1 894
New York	261	248	146	149	89	77	51	42	23	8	1 094
New Jersey	45	49	35	35	13	16	10	6	1	2	212
Pennsylvania	114	95	115	79	78	42	15	18	18	14	588
East North Central	1 515	1 295	1 127	803	694	505	444	452	287	242	7 364
Ohio	100	191	137	41	23	11	15	9	10	9	546
Indiana	115	70	44	42	19	10	27	13	9	8	357
Illinois	554	513	514	442	445	294	243	203	140	133	3 481
Michigan	302	219	193	93	61	52	68	111	60	57	1 222
Wisconsin	444	302	239	185	140	138	91	116	68	35	1 758
West North Central	1 765	1 103	1 088	1 109	1 150	1 016	831	666	660	402	9 790
Minnesota	386	302	335	283	189	139	133	151	116	65	2 119
Iowa	902	412	377	549	767	724	556	351	405	179	5 222
Missouri	141	82	115	80	67	45	44	54	39	40	707
North Dakota	6	5	29	38	20	7	13	15	20	23	176
South Dakota	80	67	46	10	32	23	23	21	49	33	384
Nebraska	95	89	46	15	10	3	3	32	8	16	317
Kansas	155	146	120	134	65	75	59	42	23	46	865
South Atlantic	419	307	392	280	272	168	163	119	92	117	2 419
Delaware	7	2	2	0	2	0	0	1	0	0	14
Maryland	52	57	46	44	39	12	13	6	0	7	276
District of Columbia	0	3	0	0	4	1	1	0	2	0	11
Virginia	74	82	73	67	81	47	58	46	30	35	593
West Virginia	14	8	7	8	13	7	5	6	9	3	80
North Carolina	21	16	25	21	28	21	5	3	2	11	153
South Carolina	48	22	31	9	11	6	8	3	2	5	145
Georgia	136	133	122	95	84	64	63	45	35	44	821
Florida	67	74	86	36	10	10	10	9	12	12	326
East South Central	313	226	204	174	183	136	115	106	88	73	1 618
Kentucky	15	21	23	19	17	12	11	15	22	22	177
Tennessee	92	70	39	51	44	41	35	38	34	29	473
Alabama	124	74	76	43	55	37	29	18	11	11	478
Mississippi	82	61	66	61	67	46	40	35	21	11	490

TABLE 1—*Continued*

	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1947 to 1956
West South Central	640	728	545	456	279	241	207	213	137	107	3 553
Arkansas	47	44	30	34	37	32	47	40	26	30	367
Louisiana	44	37	30	32	48	23	18	49	29	28	338
Oklahoma	89	86	144	102	71	68	38	40	31	17	686
Texas	460	561	341	288	123	118	104	84	51	32	2 162
Mountain	415	401	254	132	124	80	48	84	44	29	1 611
Montana	6	4	13	18	9	9	8	7	6	1	81
Idaho	33	30	29	24	30	14	9	13	6	5	193
Wyoming	1	11	8	5	8	4	10	4	3	4	58
Colorado	268	249	99	55	39	20	8	17	12	5	772
New Mexico	3	6	8	1	0	3	2	12	3	4	42
Arizona	21	18	18	16	8	12	1	14	6	3	117
Utah	82	87	75	9	27	18	10	16	8	7	334
Nevada	1	1	4	4	3	0	0	1	0	0	14
Pacific	370	246	197	202	171	180	80	69	72	46	1 633
Washington	66	43	26	37	62	58	5	11	6	8	322
Oregon	20	39	45	45	9	7	7	10	10	3	195
California	*284*	*164*	*176*	*120*	*100*	*115*	*68*	*48*	*56*	*35*	*1 116*
Total	6 321	4 991	4 235	3 510	3 139	2 537	2 032	1 823	1 444	1 080	31 112

* Subject to slight change on final verification of cases

at large The comparison indicates that the reduction in numbers of cases was not uniform for all of the states as evidenced by a calculated χ^2 of 518 93 and a theoretical χ^2 of 56 50 with 48 degrees of freedom at the probability level of 0 01 Of those states in which the expected numbers of cases are less than the actual reported numbers the deviations for Arkansas Louisiana Kentucky and North Dakota appear significant

Since the greatest decrease in incidence of bovine brucellosis has occurred in the last 5 years the incidence and distribution of the total cases by states are compared with the data for the preceding 5 years in TABLE 3 Here again it is evident that the reduction in numbers of cases was not uniform as attested by a calculated χ^2 of 2086 47 and a theoretical χ^2 of 76 15 with 48 degrees of freedom at a probability level of 0 01 From this analysis of states reporting less than the expected number of cases the deviations may be significant for the following states Wisconsin Texas New York Pennsylvania Connecticut Indiana Nebraska Colorado Utah Ohio Florida Maryland Oregon New Jersey South Carolina and New Hampshire Wisconsin Connecticut and New Hampshire are accredited under the brucellosis eradication program Oregon Pennsylvania and Utah are expected to receive accreditation during 1958 Although the control and eradication program was organized in Texas in 1957 and an effective program is not yet operative the relative decrease in

TABLE 2

A COMPARISON OF THE INCIDENCE AND DISTRIBUTION OF NOTIFIED CASES OF BRUCELLOSIS IN THE UNITED STATES IN 1947 AND 1956

State	1947	1956	
	Number of cases	Number of cases	Expected number of cases
1 Iowa	902	179	15
2 Illinois	554	133	95
3 Texas	460	32	79
4 Wisconsin	444	35	76
5 Minnesota	386	65	66
6 Michigan	303	57	52
7 California	284	35	49
8 Colorado	268	5	46
9 New York	261	8	45
10 Connecticut	178	5	30
11 Kansas	155	46	26
12 Missouri	141	40	24
13 Georgia	136	44	23
14 Vermont	136	14	23
15 Alabama	124	11	21
16 Indiana	115	8	20
17 Pennsylvania	114	14	19
18 Ohio	100	9	17
19 Nebraska	95	16	16
20 Tennessee	92	29	16
21 Oklahoma	88	17	15
22 Mississippi	82	11	14
23 Utah	82	7	14
24 South Dakota	80	33	14
25 Massachusetts	76	15	13
26 Virginia	74	35	13
27 Florida	67	12	11
28 Washington	66	8	11
29 South Carolina	48	5	8
30 Arkansas	47	30	8
31 New Jersey	45	2	8
32 Louisiana	44	28	7
33 Maryland	52	7	9
34 Idaho	33	5	5
35 Maine	32	1	5
36 New Hampshire	24	1	4
37 North Carolina	21	11	4
38 Arizona	21	3	4
39 Oregon	20	3	3
40 Rhode Island	18	4	3
41 Kentucky	15	22	3
42 West Virginia	14	3	3
43 Delaware	7	0	1
44 Montana	6	1	1
45 North Dakota	6	23	1
46 New Mexico	3	4	1
47 Wyoming	1	4	0
48 Nevada	1	0	0
49 *District of Columbia*	*0*	*0*	0
Totals	6 321	1 080	1 072

Based on the assumption of a uniform rate of reduction

TABLE 3

A COMPARISON OF THE INCIDENCE AND DISTRIBUTION OF NOTIFIED CASES OF BRUCELLOSIS FOR THE PERIODS 1947 TO 1951 AND 1952 TO 1956

State	1947 to 1951	1952 to 1956	
	Number of cases	Number of cases	Expected number of cases
1 Iowa	3 007	2 215	1 208
2 Illinois	2 468	1 013	991
3 Minnesota	1 515	604	609
4 Wisconsin	1 310	448	526
5 Texas	1 773	389	712
6 Michigan	874	348	351
7 California	794	322	319
8 Georgia	570	251	230
9 Kansas	670	245	250
10 Missouri	485	222	195
11 Virginia	377	216	151
12 New York	893	201	359
13 Oklahoma	497	194	198
14 Tennessee	296	177	119
15 Arkansas	192	175	77
16 Mississippi	337	153	135
17 South Dakota	235	149	94
18 Louisiana	191	147	77
19 Pennsylvania	481	107	193
20 Alabama	372	106	149
21 Washington	234	88	94
22 Vermont	227	83	91
23 Kentucky	95	82	38
24 North Dakota	98	78	39
25 Massachusetts	178	70	72
26 Connecticut	419	69	168
27 Indiana	290	67	116
28 Nebraska	255	62	102
29 Colorado	710	62	285
30 Utah	275	59	110
31 Ohio	492	54	198
32 Florida	273	53	110
33 Idaho	146	47	59
34 North Carolina	111	42	45
35 Maryland	238	38	96
36 Oregon	158	37	63
37 Arizona	81	36	33
38 New Jersey	177	35	71
39 Montana	50	31	20
40 West Virginia	50	30	20
41 Wyoming	33	25	13
42 New Mexico	18	24	7
43 South Carolina	121	24	49
44 Maine	72	16	29
45 Rhode Island	41	13	16
46 District of Columbia	7	4	3
47 New Hampshire	39	3	16
48 Nevada	13	1	5
49 Delaware	13	1	5
Totals	22 196	8 916	8 916

Based on the assumption of a uniform rate of reduction

notified cases in Texas is greater than in Wisconsin For states reporting more than the expected numbers of cases the deviations may be significant in the following instances Iowa Virginia Tennessee Arkansas South Dakota North Dakota Alabama Louisiana Kentucky and New Mexico No states in this list have received accreditation New Mexico is expected to be accredited during 1958 The data for Iowa probably reflect the relative importance of swine brucellosis in the epidemiology of the human disease in that state In Minnesota which has been accredited and Michigan which is probably due for accreditation in 1958 the reported cases are approximately the numbers expected States not previously mentioned that have received accreditation are North Carolina Maine Washington Delaware and Vermont those probably soon to be accredited are Rhode Island and Montana

As yet no clear relationship is apparent between the reduction in incidence of notified cases of brucellosis and the degree of control and eradication achieved in the various states Perhaps in the next several years a real effect will be noted It should be mentioned that in states with the more effective programs greater numbers of infected cattle are moved to slaughter This may result in a temporary increase in exposure of occupational groups handling these animals

As already stated the notified incidence of the disease may be entirely too low Epidemiological studies offer another means of evaluating the incidence *Brucella* infection usually results in specific dermal hypersensitivity to *Brucella* antigen Repeated injection of *Brucella* antigen into control subjects does not produce this sensitivity[10, 11] nor does the ingestion of dead organisms[1, 13] Presumably growth of the organism within the host is necessary for sensitization to occur Persons exhibiting dermal hypersensitivity to *Brucella* antigen are regarded as having been infected at some time From 10 to 25 per cent of the general population in the United States varying in different areas of the country exhibit specific allergy for *Brucella* therefore they must be assumed at one time to have been infected with this agent If one considers this incidence in the light of the notified incidence of brucellosis in man it is difficult to reconcile the two Probably many of these individuals remain asymptomatic and never develop clinical disease Even so one cannot avoid the conclusion that a great deal of clinical illness due to *Brucella* escapes recognition and does not appear in the reports of notified cases In recognition of this discrepancy there have been a number of attempts to estimate a more realistic incidence than that reported Difficulties arise because of the duration of the disease One estimate placed the number of persons in the United States suffering from *Brucella* infection during any particular year at between 40 000 and 4 000 000 (4000 cases were officially reported)[14] A conservative estimate for the year 1949 based upon data derived from a few centers regarded as providing reliable information by isolation of the organism from a high proportion of patients and by employment of an adequate serologic test extrapolated on a national basis placed the actual incidence at approximately 2½ times the notified incidence[15]

Epidemiology

Brucellosis in man is mainly an endemic disease of sporadic occurrence. Man contracts the disease by consuming unpasteurized milk or other dairy products derived from infected animals or by direct contact with infected animals or their tissues. The only substantiated cases of transmission from human to human are from mother to nursing infant (M. K. Castaneda, Hospital General, Mexico D. F., Mexico, personal communication).

While brucellosis has been described in many animal species, those of primary epidemiological importance for man are cattle, swine, goats, and sheep. *Br. abortus* is found primarily in cattle, *Br. suis* in swine, and *Br. melitensis* in goats and sheep. However, these species are not confined strictly to their respective main hosts. Goat-borne disease due to *Br. melitensis* has existed in the United States for many years in the states along the Mexican border.[16, 17] There have been few other foci of infected goats, the most noteworthy in Colorado in 1946,[18] where a considerable number of human cases occurred. The infected goats were destroyed and, at present, *Br. melitensis* infection in goats is believed to occur only in the southwestern states in low incidence. The disease in sheep has been nonexistent in the United States, perhaps because we do not produce milking sheep. The only important sources of the disease for man are cattle and swine. Human disease derived from infected cattle has been present throughout the entire country. Disease contracted from swine has been more limited in geographical extent and has been confined mainly to hog raising and hog slaughtering areas.

Figure 2 presents the total reported cases by states for the period 1927 to 1956. Six states accounted for the bulk of the reported cases. There is a marked correlation between the numbers of cases, the sites of large packing plants, and the distribution of farm animals.[19] Since most milk now sold in trade is pasteurized, milk-borne brucellosis is largely confined to the rural areas. Brucellosis in the United States has become almost entirely an occupational disease[19, 20] of those who contact infected animals or their tissues, that is, *farmers, dairymen, packing plant workers, butchers, veterinarians, and laboratory scientists*. Epidemiological studies done in 1949 show that in 75 per cent of proved cases of brucellosis there was a history of direct contact with infected animals or their tissues.[21] Only 25 per cent of cases were judged to be due to drinking unpasteurized milk. Spink[22] states that only 10 per cent of his current cases fail to give a history of direct contact and are presumed to be due to the consumption of unpasteurized dairy products.

While brucellosis in cattle is due mainly to *Br. abortus*, the other species may infect this host and be transmitted to man. *Br. melitensis* has been isolated but seldom from cattle in the United States. *Br. suis*, however, has been found in infected cattle and many of the reported milk-borne epidemics have been due to this species.[23-26] *Br. suis* infection does not seem to spread readily in cattle and currently bovine brucellosis due to this species is believed to be of little epidemiological importance.

With the reduction in milk-borne disease, swine have assumed relatively

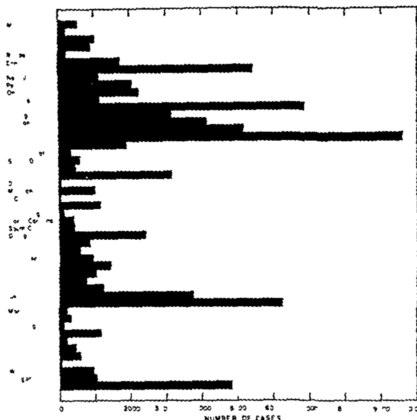


FIGURE 2 Cumulative notified cases of brucellosis in the United States from 1927 to 1950

greater importance in the epidemiology of human brucellosis While most clinical disease in swine is attributable to *Br suis* both of the other species infect these animals In 1946 *Br melitensis* was found infecting hogs in Iowa and cases of human brucellosis due to this species were attributed to this source[77] There have been continued reports of cases from Iowa Minnesota and elsewhere The clinical disease in swine resembles that caused by *Br suis* In culturing the mandibular lymph glands of 5000 marketed hogs we isolated both *Br melitensis* and *Br abortus* as frequently as *Br suis*[29] This was the first report of the recovery of *Br abortus* from naturally infected hogs Meat inspectors who worked exclusively on the hog kill and who developed brucellosis were found to be infected with *Br abortus* and they presumably contracted this infection from hogs Similarly human cases of *Br melitensis* infection were found in persons working only on the hog kill Spink also reports epidemiological evidence incriminating the hog as a source of *Br abortus* infection

of man.[30] The infection in swine due to *Br. abortus* seems limited in clinical manifestations and in infectivity for other swine. However, epidemiologically it is of importance since persons who come in contact with the tissues of infected swine may develop infection caused by this species.

While the control and eradication program for bovine brucellosis has brought about a marked reduction in the number of infected cattle in the country and while further reduction is anticipated, it should be realized that even when all the states receive accreditation, some infection in cattle will still exist and occupational groups will still be exposed. Many factors seem to be involved in determining whether or not man contracts this disease. Perhaps the host factors are the most important. Even though the number of infected animals may be less, persons working in exposed environments will still come in contact with such animals and their tissues repeatedly; those individuals who are susceptible may be offered adequate exposure to contract the disease. It is anticipated that brucellosis in man due to *Br. abortus* contracted from cattle will decline to a low figure and then remain static. Furthermore, there has been no serious effort to eradicate swine brucellosis and this source of infection for man continues to be of importance. At present we cannot foresee the day when brucellosis in man will cease to occur in the United States.

Diagnosis

In view of the vagaries of the organism and of the disease, the diagnosis of brucellosis in man can be most difficult. In the acute disease the symptoms resemble those of many other acute infections; in the chronic phase they may be multiple, vague, and varied. There are no pathognomonic signs. The clinician cannot make a clinical diagnosis with any degree of assurance; a definitive diagnosis is dependent upon the results of laboratory tests. Factors relative to culture of the organism and application of the agglutination test have already been discussed. With the development of better culture techniques and an increased understanding of the organism and of the nature and course of the infection in man, sound principles of diagnosis have emerged.[31,32] These have been well summarized by the Committee on Brucellosis of the National Research Council, Washington, D. C. In the second report of this committee, "Diagnostic Criteria for Human Brucellosis,"[32] it was recognized that the only proof of the diagnosis lies in the isolation of the organism from the patient, and that in most acute cases of the disease this is possible. In the absence of cultural proof, it was considered that an agglutination titer of 1:320 or higher, or a rising titer, offered presumptive confirmation of a clinical diagnosis. It was further recognized that tests to determine dermal sensitivity to *Brucella* antigen provide no information useful to the clinician in diagnosis and it was recommended that they should not be used for this purpose.

Since bacteremia is often intermittent, success in isolating *Brucella* from human blood may necessitate a series of cultures. The organism often may be isolated from bone marrow or lymph nodes removed for culture.

When attempts at culture fail to yield the organism, dependence must be placed on the results of an agglutination test. Since the advent of the broad

spectrum antibiotics many patients have been treated with these drugs prior to the consideration of the possibility of brucellosis or before referral to a diagnostic center The disease is partially suppressed and the organism less readily isolated from the blood the agglutination test assumes increasing importance in diagnosis

If dependence must be placed on a serologic test it is evident that a standard antigen and a standard technique for performing the test are most necessary to provide accuracy and uniformity in diagnosis The need for and choice of a standard antigen were considered by the National Research Council Committee and these deliberations led to Report No 3 A Standardized Antigen and Agglutination Technic for Human Brucellosis [34] An antigen made from *Br abortus* Strain 1119 prepared according to the method used at the Animal Disease Station Agricultural Research Service United States Department of Agriculture Beltsville Md for preparation of the antigen used in veterinary medicine was recommended as the standard A tube test with incubation at 37 C for 48 hours was specified The technique and reading of the test were described in detail

The development of these criteria for diagnosis and of a standard agglutination test are important accomplishments and should promote greater accuracy and more uniformity in the diagnosis and reporting of brucellosis

The greatest remaining problem in diagnosis is posed by chronic brucellosis There are two situations that are very difficult one presented by the patient who first comes to medical attention during the chronic stage of the disease when laboratory tests are less helpful the other by the patient with a past history of proved brucellosis in whom all signs of the disease have cleared but who continues to have subjective complaints for months or years

Therapy

The principles of therapy in brucellosis have emerged slowly and painfully Prior to the days of antibiotics dozens of remedies were enthusiastically proposed and later found wanting Among these were various vaccines that are still widely used although convincing evidence of their usefulness has not been furnished The specific therapy of the disease dates from 1946 with the introduction of a combination of streptomycin and sulfadiazine [35-37] While this combination was effective and constituted a marked advance in therapy it proved fairly toxic [38] and the relapse rate was considerable This treatment fell into disrepute with the introduction of the broad spectrum antibiotics These drugs were found to suppress the clinical manifestations of the disease but the relapse rate was high A combination of dihydrostreptomycin and chlortetracycline [39, 40] has proved effective with or without the addition of sulfadiazine or triple sulfonamide [41-43] I prefer this latter combination In growth inhibition bactericidal and manometric studies these drugs have been shown to act synergistically [44] They are highly effective against *Brucella in vitro* but when the organisms are contained inside cell their action becomes mainly suppressive rather than bactericidal [45] Any of the broad-spectrum antibiotics may replace chlortetracycline in this combination

of man.[30] The infection in swine due to *Br. abortus* seems limited in clinical manifestations and in infectivity for other swine. However, epidemiologically it is of importance since persons who come in contact with the tissues of infected swine may develop infection caused by this species.

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Since 1949 I have used dihydrostreptomycin (2 gm per day) chlortetra cycline or currently tetracycline (2 gm per day) and sulfadiazine or triple sulfonamide in a dosage to yield a blood level of 10 mg per cent in the treatment of proved cases of brucellosis No case has relapsed or failed to recover from the disease In the uncomplicated case treatment is continued for 3 weeks and in chronic cases or in those with complications, for a longer period

In formulating a logical approach to the therapy of brucellosis it should be realized that in most patients it is a self limiting disease with spontaneous recovery occurring within a few weeks or months Evaluation of the effective ness of therapy can be most difficult The more important principles of treat ment may be summarized as follows

(1) There should be prompt and accurate diagnosis

(2) Bed rest is always indicated, many patients will recover on bed rest alone

(3) The physician should search for local lesions that may necessitate longer medical treatment or definitive surgical treatment, in any case of more than a few weeks duration a Roentgen survey of the skeleton should be made to detect osteomyelitic lesions

(4) Appropriate specific antibiotic therapy should be employed

(5) As the best current therapy is mainly suppressive treatment should be continued for a sufficient period to allow the defenses of the body to overcome the infection

(6) The patient should have graded activity and should undergo careful observation during convalescence to avoid relapses

(7) Sympathetic and intelligent attention should be given to the psychiatric aspects of the disease since many patients develop depressions suicidal ten dencies and even psychoses

(8) There should be adequate diet and supportive measures as indicated

(9) The physician should have a strong reluctance to make a diagnosis of psychoneurosis when the possibility of brucellosis exists

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BRUCELLOSIS IN LIVESTOCK CONTROL AND ERADICATION

By C K Mingle

Agricultural Research Service United States Department of Agriculture Washington D C

Brucellosis of livestock has been recognized as a serious problem in the United States for almost a half century However it was not until 1934 that organized efforts were made to combat the disease in cattle In that year the cooperative state federal bovine brucellosis eradication program was launched as part of a cattle reduction plan that was brought about as a result of drought conditions in many sections In the beginning it was estimated that approximately 10 per cent of the adult cattle in the United States were affected with brucellosis

In the years prior to 1947 the program was greatly retarded by a lack of uniformity in the procedures employed in different parts of the country As a means of correcting this deficiency the former Bureau of Animal Industry Washington D C took steps to encourage the establishment of uniform control and eradication practices In time sufficient interest was developed along these lines to bring about the adoption of recommended uniform bovine brucellosis eradication procedures at the December 1947 meeting of the United States Livestock Sanitary Association held in Omaha Nebr These recommendations were designed to provide the flexibility necessary for handling brucellosis problems under varying herd conditions and still maintain a standard level of uniformity The resulting Uniform Methods and Rules have contributed much to the progress achieved in recent years Basically the current program is built around 4 control and eradication plans Plan A test and slaughter with or without calf vaccination Plan B test calf vaccination temporary retention of reactors Plan C calf vaccination without test of any part of the herd and Plan D adult vaccination

Plans B C and D are considered temporary procedures to be used only in herds where heavy infection exists until Plan A can be put into operation without serious economic shock Plan A on the other hand has eradication as its immediate goal and is the method of initial choice wherever possible

The Uniform Methods and Rules also provide for the establishment and maintenance of Modified Certified Areas in which the animal and herd infection rates do not exceed 1 per cent and 5 per cent respectively It has been shown conclusively that the increased protection afforded by operating a program of this type on an area basis is essential in maintaining the gains made through field operations

Bovine Brucellosis Eradication—Progress from 1934 to 1954

During the first 20 years of the bovine brucellosis eradication program a total of nearly 11 million herd representing approximately 127 million cattle were blood tested for brucellosis The indicated animal infection rate over this same period was reduced from about 10 per cent to 2 6 per cent In the fiscal year 1954 infection was disclosed in 14 2 per cent of the herds tested

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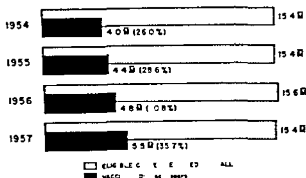


FIGURE 3 Official calf vaccinations Symbol M = million Reproduced by permission of the Agricultural Research Service

ties carried out during the first three fiscal years of the accelerated program (1955 through 1957)

Blood testing Over this period a total of 46.8 million cattle in 3.3 million herds were blood tested for brucellosis Of this number 2 2 per cent of the cattle and 12 8 per cent of the herds tested showed evidence of infection In comparison with the preceding 3-year period this represents an increase of 63 2 per cent in the number of herds tested a 92 4 per cent increase in cattle tested and a reduction of 3 per cent and 1 2 per cent in the respective infection rates

Brucellosis ring testing Remarkable increases also have occurred in milk and cream ring testing during the past 3 years and the reduced percentages of ring-suspected herds disclosed are significant of the progress being made Over this period a total of 4 7 million herds were tested of which 15 6 per cent were classed as suspicious These figures represent an increase of 133 1 per cent in the number of herds tested and a reduction of 11 4 per cent in the number of suspicious herds disclosed as compared with the preceding 3-year period Although the brucellosis ring test has recognized imperfections there is increasing evidence that it is proving to be one of the most valuable tools we possess for combating brucellosis in dairy sections of the country

Vaccination In common with testing operations vaccination has increased also each year during the accelerated phase of the program For the fiscal years 1955 19 6 and 1957 a total of 14 6 million official vaccinations was recorded This compares with 10 8 million reported for the preceding 3 years and represents an increase of 34 9 per cent It has been interesting to note the extremely low incidence of infection detected in areas where vaccination has been practiced on a wide scale over the past few years Many vaccinated areas in which high infection rates were known to have existed now show surprisingly low levels of infection

Area certifications Strong encouragement has been given in the accelerated campaign to the development of Modified Certified Brucellosis-Free Areas As explained before this designation signifies that the infection rates in such

Although Strain 19 vaccination was not officially adopted until 1941 there were more than 21 million calves vaccinated during the following 14 years The use of vaccine has increased consistently each year since it became an integral part of the program While it is impossible to evaluate accurately the benefits derived from vaccination there is increasing evidence that it has played a very important role in reducing the previously high incidence of infection in certain areas of this country The importance of providing a serviceable degree of protection in otherwise susceptible cattle will continue until complete eradication of the disease is accomplished

The Accelerated Bovine Brucellosis Eradication Program

Through additional funds made available by the Eighty third Congress it was possible in 1954 to expand the cooperative state federal campaign to eradicate brucellosis from cattle All phases of the program were vastly increased over the previous levels FIGURES 1 2 and 3 graphically illustrate the activi

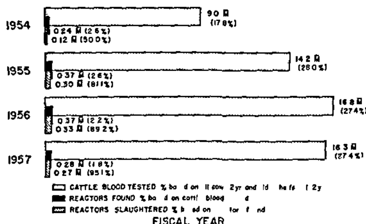


FIGURE 1 Bovine brucellosis Official blood serum agglutination tests Symbol $\overline{M}$ = million Reproduced by permission of the Agricultural Research Service

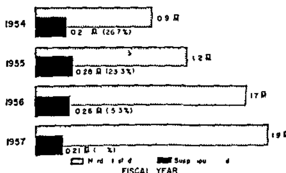


FIGURE 2 Official brucellosis ring tests Symbol $\overline{M}$ = million Reproduced by permission of the Agricultural Research Service

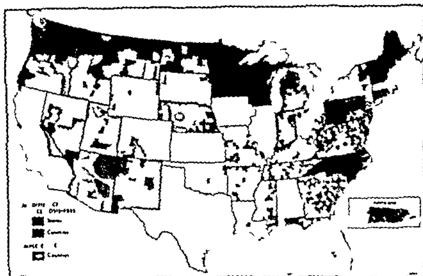


Figure 5 Brucellosis eradication program area certification status as of June 30 195 Reproduced by permission of the Agricultural Research Service

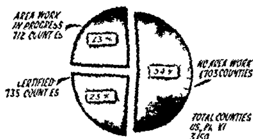


Figure 6 Brucellosis eradication program certified and area work counties in the United States Puerto Rico and the Virgin Islands as of June 30 1957 Reproduced by permission of the Agricultural Research Service

Many states have set 1960 as the goal for certification Although this objective will not be attained in all cases it does not appear nearly as visionary as when that date was designated a few years ago The enthusiasm and support for the eradication of brucellosis on the part of the livestock industry has never been greater than at present If the momentum generated over the past few years can be maintained by adequate financing for another five or six years all of the United States should be certified by that time

As progress toward certification is accelerated additional attention must be given to other known reservoirs of infection namely swine goats and sheep At present we are considering plans that it is hoped will lead to pilot field

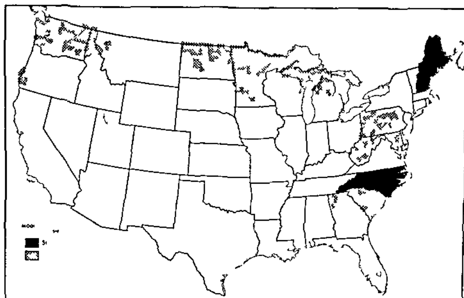


FIGURE 4. Brucellosis eradication program area certification status as of September 30, 1954. Reproduced by permission of the Agricultural Research Service.

areas do not exceed 1 per cent for animals and 5 per cent for herds. FIGURES 4, 5, and 6 depict the advances made during the past 3 years in qualifying such areas. As illustrated in the FIGURE 4 map, there was a total of only 334 counties, including 3 entire states, recognized as Modified Certified Areas at the time the accelerated program became operative on October 1, 1954. In contrast, FIGURE 5 shows a total of 735 counties, including 7 complete states, qualified as certified areas on June 20, 1957. It also depicts an additional 712 counties operating on a complete area basis leading directly toward early certification. During the past fiscal year new initial county certifications have been recorded at the rate of about 20 each month. As indicated on FIGURE 6, there were 735 certified counties and 712 other counties conducting complete area work as of June 30, 1957. Thus, of a total of 3150 counties in the United States, Puerto Rico, and the Virgin Islands, 46 per cent were either currently certified or rapidly approaching that status.

Future Outlook

Since the time data were assembled for the fiscal year 1957, the entire states of Vermont and Connecticut and 73 counties elsewhere have been added to the list of certified areas. This makes a total of 808 counties, including 9 complete states, so qualified as of September 1, 1957. It is expected that at least 800 additional counties, including 5 more states, will achieve certification during the current fiscal year. This would result in a total of 14 states and about 50 per cent of all domestic and territorial counties being certified by June 30, 1958. It is anticipated also that by the same date most if not all states will be conducting complete area programs designed to achieve early certifications.

all interested groups By maintaining the pressures developed over the past few years through adequate financing from state and federal sources we should reduce the incidence of bovine brucellosis throughout the United States to 1 per cent or less within the next five years and the brucellosis threat from this source eventually should be eliminated

Discussion of the Paper

OSCAR SUSSMAN (*Bureau of Veterinary Public Health State of New Jersey Department of Health Trenton N J*) C K Mingle in his use of the editorial we undoubtedly meant to include the public health authorities local state and national who were of considerable help in the preparation of the effective control of brucellosis or even of its eradication He indicated that it was gratifying to him to note that in the last few years *Brucella* infected cattle were moving out of the farm herds and into the slaughterhouses where they could no longer infect other cattle

In this connection it is worthy of mention that the action of the Board of Health of Chicago Ill gave considerable incentive to the State of Wisconsin in its determination to do an effective job in its attempt to eliminate brucellosis Several years ago the health authorities of Chicago indicated that they would not continue the purchase of milk from areas in which the cattle were infected with this disease even if the milk were pasteurized Subsequently similar action was taken by the public health authorities of several other areas including the State of New Jersey Department of Health which set a deadline beyond which the importation of milk from such areas would be prohibited thereafter no milk could be sold for human consumption except from animals that were known to be free of brucellosis

Regulations statutes and codes of this type have not been and could not be introduced and enforced by the agricultural authorities until such time as the public health authorities gave them the needed administrative support and created an economic incentive to the dairy farmer to cooperate if he wished to sell his product

Mingle has indicated that the agricultural authorities were unable to move *Brucella* reacting cattle off the farms after they had been identified by branding However of recent years these cattle have been moving to the slaughterhouses as already noted I submit that this exodus from the farms was initiated and sustained primarily by the public health authorities This experience repeats the history of the milk control laws that gave impetus to the tuberculosis-eradication campaign I am quite certain that Mingle and others in departments of agriculture realize that the cooperative efforts of the state and local health departments have resulted in considerable aid to farmers and to the national economy through health regulations that implement the desires of the informed segment of the agricultural interests

My remarks are made not in disagreement with Mingle but merely as additional evidence for the reader that there exists a considerable amount of valuable cooperative enterprise between the public health and agricultural authorities in their mutual concern for the control of animal diseases as they relate to human health

studies designed to determine the adequacy of existing recommended procedures for eradicating *Brucella suis* infection from swine on an area basis

Based on the available knowledge of *Brucella suis* and *Brucella melitensis* infections as they exist in the United States the control and eradication of these types of infection should not be too difficult to achieve Records assembled by the Agricultural Research Service over the past 6 years indicate the following probable incidence of brucellosis in swine and goats Of 501 160 swine tested in 57 666 herds 26 425 (or 5 3 per cent) were classified as reactors In the case of goats 1 8 per cent of 306 696 animals tested in 13 273 herds were likewise recorded as reactors Inasmuch as these data were accumulated from voluntary testing and represent a significant number of problem herds it is probable that the indicated infection rates are higher than would be true for the United States as a whole

It is important, of course to recognize the fact that certification alone does not represent complete eradication Obviously the last vestiges of infection will be the most difficult to eliminate However with continued effort and vigilance there is no apparent reason to believe that the goal of final eradication *cannot be attained in time The resulting economic and public health benefits* will fully justify and repay the effort and expense involved

Summary

Over the past 23 years the Cooperative State Federal Brucellosis Program has been responsible for reducing the incidence of bovine brucellosis in the United States from approximately 10 per cent in 1935 to 1 8 per cent in 1957

The first three years of the accelerated brucellosis eradication campaign undertaken in 1954 has resulted in more substantial progress than was accomplished in any similar period in the past

During the 3 year period ending June 30 1957 a total of 46 8 million cattle in 3 3 million herds were officially tested for brucellosis Of these 1 8 per cent of the cattle and 10 6 per cent of the herds showed evidence of infection This compares with 2 6 per cent animal and 14 2 per cent herd infection disclosed during the year preceding the inauguration of the expanded program

Extensive field experience has shown conclusively that the intelligent use of a standardized blood serum agglutination test the brucellosis ring test and Strain 19 vaccine can be employed effectively to combat bovine brucellosis on an area basis

The establishment of Modified Certified Brucellosis Free Areas is progressing at a faster rate than expected As of September 1 1957 there were 808 certified counties and 800 other counties that were actively working toward early qualification New county certifications are being recorded at an average rate of 20 per month

Although the certification of areas is an essential part of the program it is important to bear in mind that this designation is only an advanced step toward complete eradication With the recognized economic and public health benefits to be derived from this status we should accept nothing less

The bovine brucellosis eradication campaign now has strong support from

apply to the larval or adult stages or to both or to neither Thus liver flukes have a strict larval specificity but little adult specificity *Wuchereria bancrofti* on the other hand has a strict adult specificity but a low larval one occurring in a great many mosquitoes The human malarial parasites are specific to man and to anopheline mosquitoes The trichina worm has little if any host specificity among the mammals

Parasites in general are least likely to be acquired from animals that are very distantly related to man phylogenetically or that are physiologically quite different from him He has acquired few parasites from herbivores and none from horses but he shares many with both dogs and pigs Also it is obvious that the closer the physical association of an animal with man the better will be the opportunity of infection by a suitable mutant In general domestic animals are more important potential sources of parasites than are wild animals

The specialized external environmental requirements of parasites determine their geographical distribution Some cannot exist in certain climates because of unsuitable temperatures or humidity or because of lack of a suitable intermediate host Moreover some cannot gain entrance to hosts otherwise suitable because the habits of the host do not expose it to infection Thus for example the custom of cooking food prevents the infection of man with a great many species of parasites Herbivores fail to become naturally infected with the trichina worm only because they do not eat meat just as man does not often become infected with the fluke *Fasciola hepatica* because he does not eat grass

Man is a recently evolved animal who in modern times has been recorded as host for about 200 species of parasites A great many of these are of purely academic interest some are plain errors of identification but all have been or are being acquired from other animals A few were acquired from prehumans the malarial parasites the pinworms and the lice all came from man s ancestors Some such as *Bertiella* a common tapeworm of primates that is still occasionally recorded in humans and is carried by certain species of mites man may well have lost as a regular parasite after he gave up a purely herbivorous diet and took to an omnivorous one that is at about the same time that he acquired his Taenias *Physaloptera Ternidens* and *Oesophagostomum apiostomum* all common nematodes parasitizing monkeys in Africa are also in the process of being lost by man Some parasites were acquired quite early in human history from dogs swine and other animals that man domesticated others were acquired directly or indirectly in the past from wild animals and still others man continues to acquire from both of these sources

Protozoa

The relationship between the intestinal protozoa of man and those of other animals is still very obscure The chief potential pathogen of man is *Entamoeba histolytica* a form that is usually nonpathogenic living an apparently saprozoic life in the large bowel Under circumstances that are still far from clear this parasite enters the mucosa and becomes extremely pathogenic Under

PARASITES OF ANIMALS AND HUMAN DISEASE

By Thomas W M Cameron
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Parasitism is an extremely ancient mode of life and we have evidence of it from as long ago as the Devonian period It is important to realize however that parasitism has no necessary connection with disease In fact if a species of parasite is to continue to exist it is essential that it should not produce severe pathogenic effects in its host—at least not until sufficient opportunity has been given to the next generation of the parasite to gain a foothold in another host When disease is produced it is generally accidental resulting from superparasitism or from some abnormality in the normal life cycle of the host or of the parasite

Superparasitism is the result of the presence of too many parasites in a single host and it occurs when the parasite can reproduce itself without serious inhibition until overwhelming numbers prove too much for the defense mechanisms of the host This is seen particularly among the protozoan parasites such as the hemoflagellates or *falciparum* malarial parasites

This condition is seen among the helminths under quite different circumstances The worms cannot multiply within a single host and their progeny must escape to the exterior there to undergo some essential development before they can re enter the host Consequently superparasitism and parasitic disease depend on the numbers of parasites that enter the body Occasionally as in trichinosis a large number can do so simultaneously and cause an explosive often fatal result As a general rule however the accumulation of large numbers of parasites in a host is the consequence of continued exposure to infection There is a very important and distinct difference between helminthic infection and helminthic disease

Disease is not always due to excessive numbers of parasites sometimes some abnormality in the life cycle causes serious symptoms Thus the migrations of a single ascarid may occlude an important duct Similarly the larval stage of a parasite in an abnormal host may be able to survive but not develop and in surviving may produce serious lesions In general larval stages tend to have a greater pathogenicity than do the corresponding adult stages and this is particularly the case when the parasites are in an abnormal host

Parasites not only have existed for a long time but they have evolved along with their hosts and in evolving they have become more and more specialized in their environmental requirements internal as well as external

Their specialized internal environmental requirements have led to a considerable degree of host specificity that varies in type and degree among the different groups of parasites Some are so host specific that they can live only in a single species of host animal others can exist in a group of closely related animals or in a group of animals having some common physiological characteristics and still others have little host specificity This specificity may

America for example *Metorchis conjunctus* is a common parasite of the liver of fish eating mammals and when it occurs in numbers it causes a serious morbidity and may cause death It can and occasionally does occur in man but as its second intermediate host is the Common Sucker a fish rarely eaten by man it is not an important human parasite even though the fish is common and often heavily infected in the northeastern part of North America

The blood flukes of man now collectively form one of the most important causes of human disease and it is estimated that they infect about one fifth of the total population of the world The Far Eastern species has been known for many years to have numerous animal hosts and although I was able to show that *S mansoni* in the West Indies had a sylvatic host in the naturalized African green monkeys there (Cameron 1928) it was only recently demonstrated that both African species occur in a wide variety of native animals (Kuntz 1955) Man however is still the main source of his own infection both in Africa and in South America Blood flukes have existed as invaders of man for centuries but there is little doubt that irrigation has done much to increase their incidence in modern times

Blood flukes capable of developing to sexual maturity in man are absent from North America but blood flukes of birds and rodents which have a developmental cycle similar to that of the common human pathogens have larvae capable of penetrating human skin that it reaches by way of infected water These larvae die almost immediately but in dying they produce a sensitivity which when excited by subsequent exposure causes a sometimes violent and usually irritating dermatitis that is often called Swimmers Itch Such schistosome dermatitis is a common condition throughout North America and it can be contracted even in salt water

Tapeworms

The two human species of *Taenia* occur as adults in man only but although the larvae normally are found in food animals the cysticercus of *Taenia solium* can develop aberrantly in man as well as in the pig Growing as it does in an abnormal host it often develops in an abnormal situation In the pig it is confined to the striated muscles in man it is often found in the central nervous system where it can cause serious and often fatal cerebral symptoms This parasite is absent from the more northerly part of North America at present but it is still common in Central and South America as well as in parts of Europe and Asia

Dipylidium although a normal parasite of urban dogs and cats is found not infrequently in human beings often in young children As its normal intermediate host is a flea or a biting louse it is a little difficult to explain how humans become infected even when it is obvious that the original source of the infection was a pet dog or cat

Hymenolepis is shared by man with domestic rodents and is not uncommon in the warmer parts of the continent Its ability to dispense with an intermediate host facilitates infection of man by contamination of his food by mouse or rat feces

In its adult stage the genus *Echinococcus* is confined to the Canidae and to

these circumstances its normal biology is at least partly suspended and it seems probable that this phenomenon can be correlated with the relatively recent appearance of this protozoon as a parasite in man. It is common and usually nonpathogenic in Asian monkeys but it can infect South American monkeys and cause a serious morbidity. Morphologically identical species occur in a variety of other mammals and it would seem that this genus which also occurs in reptiles is a widespread parasite of vertebrates probably of long standing. This is probably true also of most of the other parasitic amoebae. There is no evidence however, that man normally becomes infected from other animals except perhaps in India, where monkeys may form a reservoir of potential human infection.

The position is even less clear with regard to the flagellate *Giardia* a universal and common parasite of man. What may be the same species is also common in dogs and other animals in whom as in man it is quite often associated with diarrhea. There is however no evidence as yet that man becomes infected naturally from a canine source although opportunities for such infection are undoubtedly common.

Perhaps the most puzzling of the intestinal protozoa are the coccidia. The genus *Eimeria* occurs in a large variety of animals but it does not affect man whereas the genus *Isospora* which has a much more restricted host range is occasionally reported from him.

The role of the hemoflagellates like that of the intestinal protozoa is also not well understood. Insofar as the two varieties of African trypanosomes that infect man are concerned there is no doubt that they were originally mutants of *Trypanosoma brucei* a parasite of antelopes that is able to live in man. There is also little doubt that they are now exclusively human parasites and although attempts have been made to implicate an antelope reservoir none has been completely successful.

The *Leishmania* group of which *Trypanosoma cruzi* should be considered a member is I believe of South American origin although it has spread to many other parts of the world. *T. cruzi* has not left the Americas although it has spread as far north as Texas. It is definitely a parasite of native South American animals (edentates and opossums) that only occasionally infects man and some of his domestic animals. The genus *Leishmania* with its various varieties is less well understood although it is known to occur in dogs and rodents and possibly in other animals in certain places. Some strains however appear to have become restricted to human beings.

The human malarias some varieties of which have been experimentally transmitted to African anthropoid apes are restricted to man and probably existed as such in prehominoids.

Trematodes

The Opisthorchid trematodes of mammals and the Heterophyid trematodes of both mammals and birds appear to have little host specificity in their adult stages. Although normal to fish eating hosts (including dogs and cats) in many parts of the world they occur in human beings only when fish is eaten raw or considerably undercooked (Cameron 1945). In northern North

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In its adult stage the genus *Echinococcus* is confined to the Canidae and to

a lesser extent to the Felidae (Cameron 1927). Its larval stage is the hydatid cyst which may develop in many species of mammals. Undoubtedly several different species exist but at least some of these have rather restricted ranges of intermediate hosts. There is reason to believe, however, that all of them can undergo larval development in human beings. Man can be infected of course only by swallowing the tapeworm eggs, and these can be acquired only from contact of food or fingers with the feces of carnivores. (It should be unnecessary to note that the cyst itself is *not* infective to man directly, and that it can be handled or even eaten with impunity. The tapeworm will not develop in man, and the sole source of human infection is carnivore feces.) The usual carnivore involved is the dog, and the usual intermediate hosts from which the dog becomes infected are domestic stock, principally sheep and swine. As a rule cattle are poor hosts, and although they often become infected and develop cysts, these cysts are very often sterile and abnormal. In Great Britain and parts of continental Europe the fox is a common definitive host, and horses frequently are infected with the cyst. In the northwestern part of North America there is a peculiar sylvatic cycle in which the wolf is the main carrier of the tapeworm, and a variety of deer, especially moose, elk, and caribou (including reindeer), are the carriers of the cyst. Indications at present are that this species, which also can infect dogs, is not the common *E. granulosus*. Indian hunters kill the deer and feed the lungs (containing the cyst) to their dogs. The dogs in turn become infected, and many villages have become grossly infected in consequence. In some parts of the Canadian northwest the human incidence is very high, and about one third of the local inhabitants are or have been infected.

In the southern part of North America the cyst is reported in swine but only rarely in man. In Canada the cyst is extremely rare in domestic stock, and human cases diagnosed in the southern part of that country have in most cases contracted the parasite elsewhere. The parasite is still common in many other parts of the world, including the Mediterranean region, Europe, Asia, Australia, New Zealand, South Africa, and parts of South America. It is one of the more important of the animal parasites transmissible to man, and it is responsible for a considerable amount of disease.

Fish tapeworms of the genus *Diphyllobothrium* are all of animal origin. In North America the species are widely distributed throughout the northern part of the continent and occur in a number of fish-eating mammals and possibly in birds. *D. latum*, which is the species most commonly reported from man, occurs in bears as well as in dogs, and there is some evidence suggesting that bears are the main reservoir from which man is ultimately infected. When it is realized that the tapeworm can produce several million eggs daily for a decade, it is easy to understand how a comparatively few reservoirs could keep the infections at a high level in fish (Cameron 1947). This is the commonest human tapeworm in North America, the infection usually deriving from uncooked or undercooked fish. Although it is known to produce some morbidity elsewhere and, by its requirements of vitamin B_{12}, to cause a serious anemia, virtually nothing is known of its pathogenicity in North America.

The final larval stages of several species of this tapeworm are capable of

living in a somatic position in man although only after actual mechanical transfer from the final intermediate host to a wound or to the orbit These pargana as they are called usually remain localized but they occasionally become malignant and metastasize as is also sometimes the case with old larval echinococci

Nematodes

The human whipworm *Trichuris trichiura* is quite common in North America but it almost always occurs in such small numbers that it is difficult to understand how it can maintain itself without an alternate reservoir It is common in swine however and it seems reasonable to believe that the pig acts as such a reservoir The egg must be exposed to the atmosphere for some weeks before becoming infective and it has every opportunity of becoming a contaminant of vegetables during this period

The genus *Capillaria* is closely related to *Trichuris* and like it is widespread in a great variety of mammals Man however has no species proper to himself although he may be infected by the *Capillaria hepatica* of rodents This parasite common in North America and elsewhere lives in the livers of rats and wild Muridae The eggs are laid there and escape only after the death of the host either by natural decomposition or via the digestive tract of a carnivore These eggs are not immediately infective and they must remain in the open for several weeks before an embryo develops Infection is presumably by ingestion of the infective egg A number of human cases are on record from North America but there is no ready means of diagnosis except by biopsy or autopsy and we have no knowledge of how common this parasite really is in man In some areas however almost one third of the domestic rats are infected

Related to these parasites is the much more serious *Trichinella spiralis*

The trichina worm is really a parasite of carnivorous animals and its original home was probably the Arctic and sub-Arctic where carnivore feeds on carnivore It is still absent from the tropics but it has become common in temperate lands where animal husbandry has converted the herbivorous pig into an omnivore and so by feeding it on pork scraps has made it a reservoir for the worm The parasite has little host specificity and it can be induced to develop in almost any mammal It is fairly common in rodents but there is no evidence that the rat enters into the pig pig or pig human cycle rather it is probable that it maintains a rat-dog or rat-cat cycle In the Arctic trichinosis is a highly important human disease depending however not on swine but on wild carnivores especially bears and walrus We have no evidence as yet that rodents play any part in the cycle although foxes both white and red as well as a great variety of terrestrial mammals are commonly infected While white whales and seals are occasionally infected the sylvatic cycle seems to involve the polar bear and the walrus Man becomes infected by eating either and in some parts of the Arctic about one third of the Eskimos are infected often seriously Elsewhere in North America infection of man is usually from pork although it originates occasionally from bear flesh While the index of infection is low in southern Canada it is high in the United States where it is estimated that about 20 per cent of the population have acquired

the parasite However, infections are usually low and the clinical manifestations light Nevertheless this nematode can be considered as the most serious of all the helminth parasites of lower animals transmissible to man

Ascaris lumbricoides has a problematical origin Although several strains of this parasite may exist, there is no conclusive evidence that there is more than one species or variety of *A lumbricoides* The parasite occurs regularly in man in swine and apparently in numerous other animals including sheep The available evidence suggests strongly that husbandry practices in temperate climates make it a predominantly porcine parasite while in warm climates hygienic conditions make it predominantly a human parasite However, it is interchangeable between man and the pig and occasionally, between sheep and other animals In northern lands where there is a fairly high incidence of very light infections in man these infections are probably usually of porcine origin in general they are much too light to depend on man as their source The reverse is probably true in the tropics where pigs are rarely infected and where the human incidence may exceed 60 per cent

The relationship of this worm to human disease is still obscure However there is some evidence that both in its larval and adult form it may cause infections which even when they are light may cause toxic nutritional and traumatic damage Heavy infections which are fortunately rare in the north cause quite serious disturbances in health

Most of the other helminth parasites of man are also accidental

Toxocara canis is the common roundworm of puppies and infection of these animals is often prenatal The female dog swallows the infective eggs and the larvae develop somatically and remain alive for a long period in a dormant condition until during pregnancy they migrate through the placenta to the fetus or to the intestine of the female In either situation it can reach sexual maturity Somatic migrations are less common in male dogs in which the worm appears to develop more normally However the relationship between this parasite and the female sex hormones of the host is still obscure Beaver *et al* (1952) have shown that somatic migrations of the larva can occur in children although the worm does not appear to reach sexual maturity in them However this parasite can produce symptoms of liver disturbance and it can remain alive for months in the liver without further development

The related *Toxocara felis* of cats also can develop in this way but curiously the very similar *Toxascaris leonina* of dogs and cats does not migrate from the intestinal tract and is not involved in the production of symptoms in man although it sometimes develops to sexual maturity in his intestine

Man s two common hookworms are specific to himself but the various hookworms of dogs and cats are sometimes able in a manner analogous to that of the parasites of Swimmers Itch to penetrate human skin and there to maintain themselves as larvae for some time They ultimately die without further development However one species *Ancylostoma ceylanicum* is occasionally recorded as a sexually mature parasite in the human intestine but it may have entered the body by the mouth rather than through the integument

Some of the other bursate nematodes that normally occur in other animals

occasionally succeed in parasitizing man This is especially true of the various species of the genus *Trichostrongylus*

However a number of reports of human infection by animal helminths are probably due to mistakes in identification Eggs of helminths (for example the liver fluke) ingested in food may be passed unchanged in the feces Similarly eggs of plant nematodes have been given specific names Even more peculiar are species such as *Mecistocirrus* a parasite of swine and cattle that has been recorded as a human parasite on the sole authority of an abattoir attendant who found the worms lying on what he thought were human feces in the abattoir Others have been recorded on the basis of the size of the egg It would be unnecessary to mention these cases were it not for the fact that such instances have a way of getting into the textbooks and remaining there in subsequent editions

Strongyloides stercoralis is a common parasite of man especially in warm countries where hygiene is poor However the status of the various species of this genus is still doubtful It is very widely distributed and many species are very similar morphologically It is a comparatively recent parasite as shown by its peculiar life cycle in the free state and man may be infected by species normally occurring in domestic animals Nevertheless it is highly probable that man is usually his own source of infection

The history of the filarid worms which are carried by blood-sucking flies is so obscure that no valid deductions are possible but because of their rather exceptional mode of transmission wild animals may well be involved as the reservoir from which man originally acquired his infections *Onchocerca* probably had a ruminant host and it may still have one in Africa *Wuchereria bancrofti* now is probably an exclusively human parasite its intermediate hosts are almost always domestic insects and recent work on the related *W malayi* suggests that an Asiatic monkey (*Macaca irus*) may have been the original host of this genus (Buckley and Edeson 1956)

Ectoparasites

Parasitic arthropods affecting man offer special problems and many of them are instrumental in the introduction of other organisms into him and can be of great importance even though they are not strictly speaking parasites of man Thus the hard ticks are nonspecific parasites mainly of mammals and reptiles and none of them parasitizes man exclusively although several are important vectors of disease organisms To a large extent the soft ticks are also nonspecific although a number of species of the genus *Ornithodorus* frequently attack man while one the African *O moubata* has quite definite associations with man and may well be considered as adapted to his habits

The Trombiculid mites which in their larval stage attack mammals and birds are equally nonspecific although quite a number of them will attach themselves to the human skin

The role of *Sarcoptes scabei* is more definite There are a number of physiological varieties of this mange mite one of which is definitely adapted to living

within the human skin Most if not all of the other varieties however, can live in the human skin for short periods but there is no evidence that any of these can become an habitual human parasite

Demodex folliculorum is still one of the outstanding puzzles of medical entomology The genus is widely distributed among animals, but there is no evidence that man can be infected from any source other than human Until the life cycle of the parasite is understood both its pathogenicity and its mode of infection will remain doubtful

Of the two species of lice infesting human beings there is little doubt that the body head species (*Pediculus humanus*) has evolved along with man from prehominoid days Nevertheless it has been found on South American monkeys but not on Old World monkeys and under rather exceptional laboratory conditions it has been reared on rabbits The pubic louse is also exclusively human No animal louse can attain more than a very transitory footing on the human skin

This is not the case with fleas In spite of the popular name of human flea applied to *Pulex irritans* no species is primarily a parasite of humans *Pulex* itself is still basically a parasite of swine and originally was probably a parasite of badgers although in parts of the world such as North Africa, it has become acclimatized to live and to breed on man It is extremely rare in North America and while not uncommon in pigstys in Europe is nearly as rare on man there as it is here The closely related *Xenopsylla* is of course a rodent flea and like the common dog and cat flea it will readily attack man *Xenopsylla* is apparently spreading in North America but the commonest fleas attacking human beings are those normally found on cats and dogs

The jigger flea apparently originating in tropical America will also attack man but it also is basically a parasite of other animals Bedbugs were probably originally parasites of bats and birds but one *Cimex lectularis* has become so domesticated that for all practical purposes it is a human parasite and is very widely distributed in all temperate and subtropical regions Its tropical relative is less exclusively a human parasite *C lectularis* itself can be reared successfully on rabbits and other laboratory animals

Larval parasitism by dipterous flies is particularly common in many parts of the world In some cases it is a facultative type of parasitism with no particular host preferences and man is often infected with these nonspecific larvae In other cases parasitism has become obligatory and host preferences have become more strict This is especially true among the Oestridae but even here man is occasionally attacked There are no strictly human species *Dermatobia hominis* in spite of its name is not a specific human parasite and is common on monkeys and other animals including all domestic animals in South America

Conclusions

Man was a late comer into the animal kingdom and he probably brought with him from his prehuman stage two or three malarial parasites his pinworms and his lice Being still quite generalized and primitive physiologically he offered a good habitat for the parasites proper to the animals with which he

surrounded himself and he easily became host to his taenias his hookworms his ascarids and his filariae The more generalized his animals (in a physiological sense) and the more closely they associated with him the more likely they were to interchange parasites and swine and dogs on occasion still act as sources of human infection Man s food habits exposed him in due course to other relatively nonspecific parasites such as most flukes and the fish tapeworms but it is quite possible that this change caused him to lose some of his parasites This applies for example to the tapeworm *Bertiella* which is still common in other primates and to which man is still susceptible His bathing habits rendered him a suitable host for various skin penetrating blood flukes

In this way man acquired his major parasites quite early in his history but the process did not cease in the early days of his development it is still going on and even now he is accidentally becoming infected with parasites from both domestic and wild animals

As mentioned above it is necessary to distinguish between infection and disease Except when accidents occur as when an ascarid worm occludes a bile duct adult helminths usually cause disease only when present in considerable numbers As helminths cannot multiply continuously within a single host infection postulates exposure to a large number of infective forms usually over a considerable length of time (*Trichinella* infection is an obvious exception to the time factor) It follows therefore that disease seldom results from adult helminth parasites that are not normal human parasites This is not true of larval stages that develop not only in abnormal situations but because of the long life span of man can often develop in abnormal ways, examples include such forms as cerebral cysticercosis larval toxocariasis and daughter hydatids In these cases as in *Trichinella* a single exposure may produce clinical symptoms This is true also of the protozoal infections because of their ability to multiply within the body

As yet there are no preventive vaccines or sera for any of the parasitic infections In many cases specific chemotherapy is available but in some of them (these include some of the more serious ones) no treatment has yet been discovered The specificity of the life cycles of these parasites however makes practical preventive measures feasible often by such simple expedients as good hygiene or the adequate cooking of food

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The jigger flea apparently originating in tropical America will also attack man but it also is basically a parasite of other animals Bedbugs were prob ably originally parasites of bats and birds but one *Cimex lectularis* has become so domesticated that for all practical purposes it is a human parasite and is very widely distributed in all temperate and subtropical regions Its tropical relative is less exclusively a human parasite *C lectularis* itself can be reared successfully on rabbits and other laboratory animals

Larval parasitism by dipterous flies is particularly common in many parts of the world In some cases it is a facultative type of parasitism with no partic ular host preferences and man is often infected with these nonspecific larvae In other cases parasitism has become obligatory and host preferences have become more strict This is especially true among the Oestridae but even here man is occasionally attacked There are no strictly human species *Dermatobia hominis* in spite of its name is not a specific human parasite and is common on monkeys and other animals including all domestic animals in South America

Conclusions

Man was a late comer into the animal kingdom and he probably brought with him from his prehuman stage two or three malarial parasites his pinworms and his lice Being still quite generalized and primitive physiologically he offered a good habitat for the parasites proper to the animals with which he

surrounded himself and he easily became host to his taenias his hookworms his ascarids and his filariae The more generalized his animals (in a physio logical sense) and the more closely they associated with him the more likely they were to interchange parasites and swine and dogs on occasion still act as sources of human infection Man s food habits exposed him in due course to other relatively non pecific parasites such as most flukes and the fish tape worms but it is quite possible that this change caused him to lose some of his parasites This applies for example to the tapeworm *Bertiella* which is still common in other primates and to which man is still susceptible His bathing habits rendered him a suitable host for various skin penetrating blood flukes

In this way man acquired his major parasites quite early in his history but the process did not cease in the early days of his development it is still going on and even now he is accidentally becoming infected with parasites from both domestic and wild animals

As mentioned above it is necessary to distinguish between infection and disease Except when accidents occur as when an ascarid worm occludes a bile duct adult helminths usually cause disease only when present in con siderable numbers As helminths cannot multiply continuously within a single host infection postulates exposure to a large number of infective forms usually over a considerable length of time (*Trichinella* infection is an obvious exception to the time factor) It follows therefore that disease seldom results from adult helminth parasites that are not normal human parasites This is not true of larval stages that develop not only in abnormal situations but because of the long life span of man can often develop in abnormal ways examples include such forms as cerebral cysticercosis larval toxocariasis and daughter hydatids In these cases as in *Trichinella* a single exposure may produce clinical symptoms This is true also of the protozoal infections because of their ability to multiply within the body

As yet there are no preventive vaccines or sera for any of the parasitic infec tions In many cases specific chemotherapy is available but in some of them (these include some of the more serious ones) no treatment has yet been dis covered The specificity of the life cycles of these parasites however makes practical preventive measures feasible often by such simple expedients as good hygiene or the adequate cooking of food

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INDUSTRIAL ANTHRAX*

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While anthrax remains a scourge in many parts of the world it has steadily declined in importance in the United States Nevertheless anthrax has become increasingly interesting for there is still much to be learned about the biology of its causative agent its transmission and pathogenesis and the mechanisms of immunity to it This presentation will summarize the major developments in the field of industrial anthrax since the appearance of the reviews of Smyth (1945) Wolff and Heimann (1951) and Steele and Helvig (1953) The term industrial anthrax refers to cases acquired in nonagricultural occupations that require contact with contaminated animal materials In the United States about 90 per cent of industrial cases (about 25 to 35 cases per year) are found in workers exposed to carpet wools goat hair, or goat skins imported from countries where anthrax is prevalent Occasional cases are traced to rarer sources such as elephant tusks and bone meal There is a very slight but definite risk associated with residence in the neighborhood of factories processing these materials for a few cases of the disease have been reported in persons living or working near them

FIGURE 1 shows the number of cases of human anthrax reported yearly to the National Office of Vital Statistics (NOVS) Washington D C since 1916 Both morbidity and mortality have shown definite declines and at the present time only 40 to 50 cases of anthrax are reported yearly (These are minimum figures since under reporting of anthrax either advertent or inadvertent is not unexpected For example from 1941 to 1956 NOVS recorded 85 cases of anthrax from one state That state has made records available to us indicating that 122 cases occurred there during the same period) As will be shown later however there is little justification for the optimistic view that this decline in morbidity will continue automatically The cutaneous form of anthrax accounts for more than 95 per cent of anthrax in man reported in this country An occasional case of inhalation anthrax or generalized (septicemic) anthrax is still reported but intestinal anthrax has never been documented in the United States *Bacillus anthracis* is extremely sensitive *in vitro* to most of the common antibiotics (polymyxin is a notable exception) and deaths from anthrax are rare at present whereas a 10 to 20 per cent mortality was expected in the

* The work reported in this paper is from the Communicable Disease Center Public Health Service Atlanta Ga The Wistar Institute of Anatomy and Biology Philadelphia Pa and the University of Pennsylvania School of Medicine and Veterinary Science Philadelphia Pa It is Contribution No 20 of Microbiology and Medicine (Wistar Institute) and was supported by a contract from the United States Army Chemical Corps Fort Detrick Md

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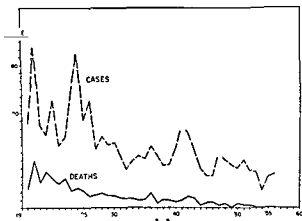


FIGURE 1. Anthrax in man in the United States, 1916 to 1956. Data from the National Office of Vital Statistics, Washington, D. C.

preantibiotic era (FIGURE 2). A high fatality rate is still associated with inhalation or generalized anthrax. Cutaneous cases in which treatment is delayed, however, are frequently more severe than those cases in which therapy is initiated within 7 days after onset.

The nationwide distribution of cases over the past several decades is shown in FIGURE 3. It is readily apparent that most of the cases are reported from only a few states. It has been realized for some time that those cases reported from the northeastern states have been primarily industrial in origin. More than 50 per cent of the 3228 cases of anthrax reported since 1916 have been from five states (Pennsylvania, New York, New Jersey, Massachusetts, and New

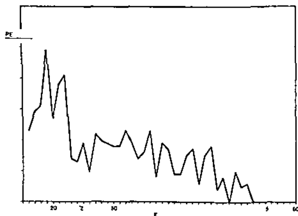


FIGURE 2. Anthrax fatality rate in the United States, 1916 to 1956. Data from the National Office of Vital Statistics, Washington, D. C.

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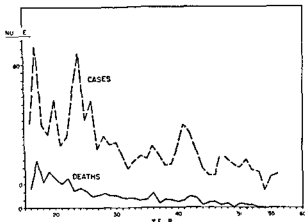


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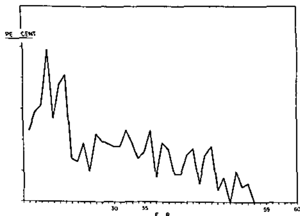


FIGURE 2 A thrax fatality rate in the United States 1916 to 1956 Data from the Na onal Offic of V t l Stat stics Washington D C

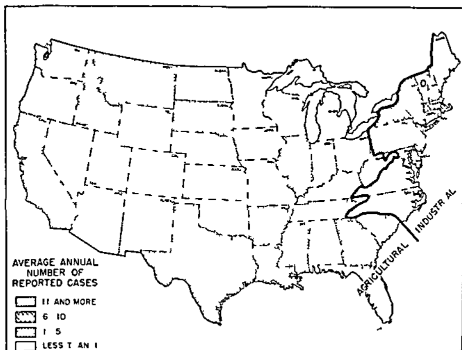


FIGURE 3 Distribution of cases of human anthrax by states 1920 to 1956 Data from the National Office of Vital Statistics Washington D C

Hampshire) which reflects the concentration of textile industries and tanneries in them Cases in other sections of the country usually have been agricultural in origin The latter patients were usually farmers abattoir workers or veterinarians who were infected as a result of handling carcasses of animals that had died of anthrax or of accidentally contaminating themselves while administering a veterinary spore vaccine Texas Mississippi California and Louisiana have accounted for more than 50 per cent of all agricultural cases

For purposes of presentation in FIGURE 3 a state was classified as a source of industrial cases when the great majority of cases reported from it have been industrial in origin even though it has occasionally reported agricultural cases South Carolina and Nevada have not reported any human anthrax since 1916 Prior to 1953 the rare cases reported from North Carolina were agricultural In 1953 a firm processing goat hair was established there and since then 9 cases have been reported among its workers Most of the anthrax reported from Virginia has been agricultural but 4 cases in that state associated with a carpet manufacturing plant were reported in 1955

Anthrax is primarily a disease of animals which constitute its most important reservoir Man is an incidental host only by virtue of contact with such animals directly or indirectly with their products As a result the incidence of human anthrax would be expected to reflect the incidence of the disease in

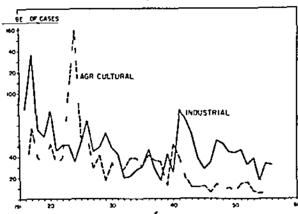


FIGURE 4 Industrial and agricultural human anthrax in the United States 1916 to 1956 Data from the National Office of Vital Statistics Washington D C

animals That this is the case is shown by FIGURE 4 which demonstrates the number of cases of human anthrax diagnosed in the United States since 1916 by type of exposure Anthrax in animals in the United States has declined since 1940 partly because of good animal husbandry and the widespread use of vaccine The incidence of human agricultural anthrax has declined correspondingly Industrial anthrax however is a reflection of the incidence of disease in foreign animals since this has not changed appreciably neither has the incidence of industrial anthrax Notable variations in the number of yearly cases of industrial anthrax can be related to changes within the industry The peaks of 1917 and 1941 probably represent the effect of wartime factors such as increased output and employment and the use of dirtier less desirable and more highly contaminated raw materials The trough in the 1930s parallels a decrease in employment and production The agricultural peak in 1924 is a reflection of epizootics in Mississippi and Texas The industrial peak in 1926 may reflect the tanning of hides from the domestic herds affected in 1924 All of these factors have become relatively stable however so that for the past 10 years about 80 per cent of the human anthrax in the United States (about 30 to 40 cases per year) can be called industrial The industries involved have been far from stable however

In the last decade there has been a marked change in the distribution of cases throughout the industries involved and some of the change can be attributed to the increased use of synthetic fibers and plastics This has particularly affected tanneries and carpet wool concerns which together contributed more than one half of the anthrax diagnosed in the United States for several decades (TABLE 1) In spite of the fact that anthrax has been declining tanneries and woolen mills now account for only 11 per cent of the cases or about 4 to 5 cases per year Cases associated with the goat hair industry now predominate their number has increased from an average of 3

TABLE 1

DISTRIBUTION OF ANTHRAX IN THE UNITED STATES BY SOURCE OF INFECTION
(Adapted from Wolff and Heiman, 1951)

Source	Percentage of reported cases traced to source				
	1919 to 1923	1924 to 1928	1929 to 1933	1934 to 1938	1955 to 1956
Hides and skins	53	38	30	35	2
Wool	3	25	27	15	9
Goat hair	12	9	4	5	65
Agriculture	13	14	26	34	21
Shaving brushes	*18*	*4*	*2*	*2*	*0*
Laboratory	—	2	3	2	0

cases per year during the 1930s to about 20 to 25 cases per year, or 65 per cent of all anthrax in 1955 and 1956. The number of persons employed in woolen mills is at least 5 times the number employed in goat hair mills.

For several years, therefore, public health workers have concentrated their efforts on the control of anthrax in the goat hair industry. The major users of goat hair are a relatively small number of firms manufacturing haircloth interlinings used to lend shape to suits and coats. This highly competitive industry is one of the last of the textile industries to withstand the inroads of synthetic fibers. It should be understood that any preventive measure that would increase the cost of their product even a few cents per pound would probably be disastrous. Consequently, protection against this disease has developed into a complicated and challenging public health problem.

The goat hair used in the United States is obtained primarily from middle eastern countries such as Pakistan, Iraq, Iran, and India. It is impossible for domestic buyers to control the disease in herds in those countries or to predict or reduce the contamination of the hair at the time of export. Raw goat hair is shipped in jute- or burlap-covered bales weighing several hundred pounds, and stevedores and truckers handling these bales have been known to develop anthrax. When the bales are opened at the factory, the material is fed into picking machines that shake the hair, separate the fibers, and remove many of the coarse impurities such as dust, rocks, bones, dried blood, and animal feces. Vacuum exhaust systems carry away much of this debris, but it is an extremely dusty operation and one would expect the risk of contracting anthrax to be great at this stage.*

The hair fibers are then fed into carding and combing machines that further classify and separate the fibers. The loose strand of hair that is produced is then blended with various other fibers such as wool or rayon to produce a rope-like material called "hair top." The top is drawn into a narrow strand and spun on frames. The resultant thread is then woven into fabric. The fabric

* In one factory the hair is scoured after it has been picked. This is done to improve the quality of the finished product rather than to prevent anthrax. This scouring process consists of feeding batches of the hair into a series of 3 vats of water and soap or soda ash at 40 to 50° C for about 30 minutes. The hair is then rinsed and conveyed to chambers in which it is dried in warm air at 80° C for 15 minutes.

TABLE 2

RELATION OF THE DEVELOPMENT OF ANTHRAX TO SURFACE CONTAMINATION AT VARIOUS STAGES IN THE PROCESSING OF GOAT HAIR 1945 TO 1956

Department	Total cases of anthrax	Cases per 100 mill employees per year				Per cent surface samples positive for *B. anthracis* 1954 to 1956
		Mill A	Mill B	Mill C	Combined	
Picking	22	4.4	4.4		4.4	66
Carding	40	4.4	4.4		4.4	62
Combing	25	4.4	2.2	4.4	4.0	59
Drawing	40	2.2	2.8	3.5	3.0	44
Spinning	100	1.4	2.3	1.8	2.2	39
Weaving	16	0.2	0.2	0.2	0.2	22
Finishing	16	0.9	0.9	0.8	0.8	19
Office	0	0.0	0.0		0.0	5
Entire mill	259	1.2	1.6	1.6	1.4	44

Excluded because of lack of reliable data.

interlining is finished by singeing (to remove extraneous hair), sizing, washing, Sanforizing, and other processes.

The danger of contracting anthrax has always seemed to be greatest in the early stages of this process. TABLE 2 summarizes information on this point collected from 3 goat hair factories. Two hundred and fifty cases of anthrax *were diagnosed in these 3 factories in the past 15 years, or 1.4 cases per 100* employees per year. The greatest number of cases came from the spinning rooms, but the highest attack rates were found in the processes preceding spinning. Attack rates averaged more than 4 cases per 100 employees per year in the picking, carding, and combing departments, or about twice that in the spinning department; relatively low rates were found in the weaving and finishing stages. The trend toward a lower risk of contracting anthrax in successive stages was noted in all the factories studied. The striking similarity of departmental attack rates in each of the factories is noteworthy. It has been suggested that the risk of contracting the disease in one of these factories is related to the proximity of the employee to the picking and carding rooms, and that *most of the plant contamination results from the spread of the organism through* the air from that source. The factories studied use similar methods of production, but their physical plants are quite different. Consequently, this striking correspondence in attack rates between factories suggests that the nature of the work is a more important determinant of the attack rate than is proximity to the hair storage and picking area. Mill C, which scours its raw hair immediately after picking, has as high an attack rate as those that do not. These attack rates may be compared to those in the wool industry furnished by Wolff and Heimann (1951), who recorded 0.078 cases per 100 employees per year in that industry, or about one twentieth the rate in the goat hair industry. The rate for workers engaged in picking, carding, and scouring wool was about 0.27 *cases per 100 employees per year, or about one fifteenth* the rate noted in the corresponding stages in the processing of goat hair. Our studies have

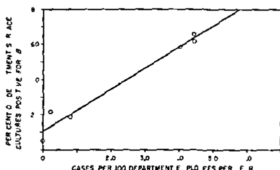


FIGURE 5 The relation of the risk of developing anthrax to the contamination of surfaces with *Bacillus anthracis* by department in three mills 1954 to 1956

shown that wool processing mills are less contaminated with the spores of *B anthracis* than are goat hair mills

TABLE 2 also summarizes the results of our studies on different occasions of the surface contamination of several goat hair mills with the spores of *B anthracis* Moist sterile cotton swabs were used to take cultures from floors and walls and from machinery and other equipment The swabs were then used to inoculate blood agar plates that were incubated for 18 hours at 37 C Colonies grossly and microscopically resembling *B anthracis* were tested for mouse virulence and lysis by the gamma bacteriophage of Brown and Cherry (1955) Forty four per cent of all samples in this series were positive for *B anthracis*, indicating a relatively high contamination of the mills with this pathogen The greatest numbers were isolated from the early dusty stages FIGURE 5 shows that the departmental attack rate in these mills bears a direct relation to the level of surface contamination

That contact by man with the organism does not necessarily lead to disease is demonstrated by the recent report of Carr and Rew (1957) who were able to recover *B anthracis* from the nose or pharynx of 14 of 101 healthy workers in 2 of these mills Again most of the recoveries were from persons working in the early stages of production While the transmission of anthrax either from infected persons or workers such as these has never been documented cases have been recorded in which asymptomatic workers might have been responsible Transmission via the clothing of these workers is another likely explanation of these incidents

It might be inferred from FIGURE 5 that if the surface contamination could be reduced to the 10 per cent level a negligible attack rate might prevail Our experience tends to confirm the importance of plant hygiene However it is probable that both the level of plant contamination and the attack rate are dependent upon the more important factor of hair contamination Attempts to decontaminate the hair have met with many difficulties In England while the establishment of the Wool Disinfection Station at Liverpool and the passage of governmental regulations have satisfactorily controlled anthrax such a plan does not seem applicable in the United States The initial cost of the equipment would be enormous it would be difficult to decide where it

should be located and the continuing costs (estimated at $3 000 000 per year) could not be absorbed by the industry. A few firms here have recently begun to steam sterilize their hair in the top stage but this is not entirely satisfactory since the procedure alters the material and makes spinning difficult. In addition persons working in the early higher risk stages are unaffected by this measure. We have evaluated several other methods of disinfection (to be reported elsewhere) and have not found them to be effective or practicable. Considering the many facets of this problem it would appear that the use of an immunizing agent to protect these workers is the most logical and practical way to reduce the incidence of anthrax. Such a material has been developed by the United States Army Chemical Corps (Wright *et al* 1954) and is being field tested in some mills.

It has been hypothesized that many workers in these factories develop immunity to anthrax by virtue of some kind of subclinical infection in this highly contaminated environment. FIGURE 6 shows one of the reasons for this view. This graph shows the number of years 46 persons who developed anthrax had worked in the industry before developing the disease. The largest number of cases for a single year was observed in the first year of employment and more than one half the cases occurred in persons who had worked less than 4 years. However it has always been difficult to explain cases occurring after 15 to 20 years of contact with this environment. In an attempt to answer this question we obtained data on the length of employment of all the workers in these factories. From this information it has been possible to estimate the composition of the exposed population over this period and to determine the relation of the length of employment to the attack rate. The graphic distribution of the length of employment of all workers (FIGURE 6) was similar to that of the

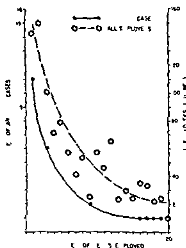


FIGURE 6 The relation of length of employment in a goat hair mill to the development of anthrax

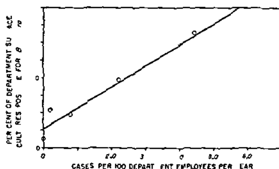


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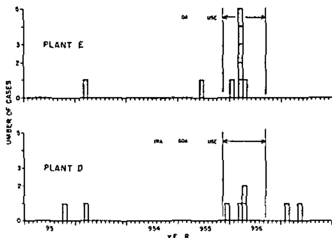


FIGURE 7 Anthrax in two related goat hair mills

it was possibly more contaminated Changes were made in the method of steam sterilization so as to ensure better penetration of steam to the center of the ball of hair top to reduce the possibility of error Plant E was again washed with disinfectant Since that time occasional cases have been diagnosed at Plant D (which uses unsterilized hair) but no further cases have been noted at its subsidiary In November 1956 3 / per cent of the surface samples taken at the subsidiary plant were positive for *B anthracis* If this low level of contamination can be maintained the number of cases of anthrax diagnosed there will probably be small

Summary

The number of cases of anthrax in humans in the United States has steadily declined to the present level of 40 to 50 cases per year Practically all diagnosed anthrax is now the cutaneous form and deaths from the disease have been rare since the introduction of antibiotics The majority of cases reported are industrial in origin and they can most frequently be traced to contact with imported goat hair The further reduction of the incidence of anthrax presents many problems but adequate ventilation and high standards of cleanliness in the mills seem to play an important role in the control of anthrax The risk of contracting anthrax is greatest in the early steps in the processing of goat hair and is greater than the risk associated with wool processing Prolonged exposure to the organism does not appear to lead to the development of subclinical infection or immunity to anthrax

Acknowledgment

We are greatly indebted to Ida Sherman of the Communicable Disease Center for furnishing statistical advice and data on the incidence of anthrax Much of the material discussed in this paper could not have been obtained

TABLE 3

RELATION OF THE LENGTH OF EMPLOYMENT TO THE DEVELOPMENT OF ANTHRAX IN FOUR COMPANIES PROCESSING GOAT HAIR 1945 TO 1956

Number of years employment	Cases of anthrax per year	Number of employees	Attack rate cases per 100 employees year
0 to 4	9.8	546	1.8
5 to 8	3.4	262	1.3
9 to 12	1.9	209	0.9
13 to 16	1.1	119	0.9
17 to 20	1.1	90	1.2
Entire group	17.3	1226	1.4

anthrax cases for the majority of workers had been employed in this industry less than 5 years. TABLE 3 shows the attack rates for varying periods of employment. These rates ranged from 1.8 cases per 100 employees per year in the first 4 years to 0.9 for those who had worked for 9 to 16 years. The rate for 17 to 20 years employment was 1.2 cases per 100 per year. None of these rates are significantly different. In addition they show much less variation than was observed between departments in these plants (TABLE 2). It was our conclusion, therefore, that these workers do not develop subclinical infection or immunity to anthrax by prolonged exposure to the organism. Actually, while a second attack of anthrax in the same individual is very rare, there are reports of its occurrence (Gold, 1946) and immunity to anthrax in humans has never been demonstrated conclusively.

FIGURE 7 outlines a small epidemic of anthrax that illustrates some of the problems already described. It charts the occurrence of anthrax at one company (Plant D) and its subsidiary elsewhere (Plant E). Picking, carding and combing are performed at Plant D, and then part of the hair is sent to Plant E for further processing. Plant E was established early in 1953 and a few months later the first case of cutaneous anthrax was reported there. An investigation revealed extensive contamination of the plant environment with *B. anthracis*. The plant was closed and thoroughly cleaned with 5 per cent formaldehyde. A check of environmental contamination following this did not reveal any *B. anthracis*. At the same time Plant D began to steam sterilize the hair top before sending it to the subsidiary. However, a second case of anthrax was reported from Plant E in June 1955. At that time 43 per cent of the environmental cultures there were positive for *B. anthracis*. In addition, it was realized that the steam sterilization had not been completely effective since *B. anthracis* was recovered from the inside of several balls of hair top shipped to Plant E. In December 1955 and in the early part of 1956 an unusual number of cases were reported from both mills: 7 cases were diagnosed at Plant E and 4 at Plant D. At that time 70 per cent of the surface samples at Plant D and 28 per cent of the surface samples at Plant E were positive for *B. anthracis*. This is probably related to the fact that the company began to use goat hair from Iran late in 1955. The management felt that the Iranian hair was "dirtier" than the hair normally used and that accordingly

NEWCASTLE DISEASE*

By R P Hanson
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Newcastle disease is an infectious highly contagious disease that afflicts chiefly chickens and turkeys Many species of birds as well as various mammals including man may contract the infection with serious consequences In birds Newcastle disease may vary in its manifestations from an acute fulminating form to subacute mild or clinically inapparent forms manifesting various degrees of generalized and systemic involvement especially of the respiratory and nervous systems It has been recognized in man chiefly as an acute transient conjunctivitis with or without malaise preauricular adenitis fever and chills Generalization of the infection with presence of the virus in the blood urine and respiratory tissues and secretions has been encountered

Properties of the Virus

In some of its characteristics the causative virus of Newcastle disease is similar to the viruses of influenza and mumps This virus is admirably suited to survival in nature as it is resistant to the adversities of environment including temperature putrefaction drying moisture and light that it encounters in the habitat of its most common host domestic poultry The virus of Newcastle disease survives long exposure to a wide range of *p*H values—extremes of 4 to 10 at 4 to 6 C for 1 week or more—that are inimical to most viruses (Moses Brandly and Jones 1947) In studies of heat stability involving 31 strains of Newcastle disease virus Hanson (1949) found that all strains remained infective after 15 min at 56 C 3 were innocuous after 30 min and of the remainder 3 retained their infectivity after such heating for 180 min

Early work by Doyle (1927) and later that of others showed that drying of Newcastle disease virus infected secretions and tissues prolonged the survival time by months or years H E Rhoades (personal communication 1957) found that strain KD NJ suspended and lyophilized in horse serum remained viable at room temperature (22 to 30 C) for 1 year in the refrigerator (6 to 7 C) for 10 years the latest period tested Another strain 11 914 lived for 1 year but not for 2 under each condition Seventeen field strains were also viable after storage in the refrigerator (6 to 7 C) for 10 years

Definitive data on the effect of light are limited Exposure of infected organ suspensions to direct sunlight for 1 hour had no effect on the virus (Iyer 1943) Exposure of virus bearing fluid to short ultraviolet rays (1600 to 1800 Å) inactivated the strains studied in 0 8 to 1 08 seconds (Brandly *et al* 1946a)

* The literature on Newcastle disease has become so voluminous that references in this paper have been limited largely to reviews and to the selected reports that are available to the authors

without the generous cooperation of plant managers and public health officials especially Cleon J Gentzkow (Pennsylvania State Department of Public Health), Forrest Bumford (New Hampshire Department of Health), Milton Werrin and A LaBocetta (Philadelphia Department of Health), Alice Broadhurst (Massachusetts Department of Labor), Herman Gold Chester, Pa and Martin Hines (North Carolina Department of Health) The assistance of Alexander D Langmuir is gratefully acknowledged Leighton Cluff Ivan L Bennett, and C Brooke Worth assisted in the preparation of this manuscript

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Discussion of the Paper

OSCAR SUSSMAN (*Bureau of Veterinary Public Health State of New Jersey Department of Health Trenton N J*) There was a known case of anthrax originating from bones imported from abroad these bones were utilized in the production of gelatin in a plant located in New Jersey Apparently the procedure used for the production of this gelatin did not kill all of the anthrax spores This is a case in which known disease organisms are present in a product that will be used as human food My question is whether or not there is considerable danger of air contamination of the finished product which is made in the same plant in which the original infected bone material was processed?

PHILIP SIGMUND BRACHMAN The question as to whether there is considerable hazard in air contamination of the finished gelatin product is related directly to the degree of contamination of the processed dried bones to the types of equipment used and to the ventilation of the plant as well as to the stages in the manufacturing process at which the actual grinding and mixing take place These factors will be all important in determining the type of aerosol that may be established in such a plant Certainly if conditions are favorable it would be possible for the finished gelatin to be contaminated with bacteriological organisms originating from the raw material via the air However one cannot hazard a guess as to the probability of such an occurrence without more information about the plant about the raw material and about other factors already cited

NEWCASTLE DISEASE*

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Newcastle disease is an infectious, highly contagious disease that afflicts chiefly chickens and turkeys. Many species of birds as well as various mammals, including man, may contract the infection with serious consequences. In birds Newcastle disease may vary in its manifestations from an acute fulminating form to subacute, mild, or clinically inapparent forms manifesting various degrees of generalized and systemic involvement, especially of the respiratory and nervous systems. It has been recognized in man chiefly as an acute, transient conjunctivitis with or without malaise, preauricular adenitis, fever and chills. Generalization of the infection, with presence of the virus in the blood, urine, and respiratory tissues and secretions, has been encountered.

Properties of the Virus

In some of its characteristics the causative virus of Newcastle disease is similar to the viruses of influenza and mumps. This virus is admirably suited to survival in nature, as it is resistant to the adversities of environment, including temperature, putrefaction, drying, moisture, and light that it encounters in the habitat of its most common host, domestic poultry. The virus of Newcastle disease survives long exposure to a wide range of pH values—extremes of 4 to 10 at 4 to 6° C. for 1 week or more—that are inimical to most viruses (Moses, Brandly, and Jones, 1947). In studies of heat stability involving 31 strains of Newcastle disease virus, Hanson (1949) found that all strains remained infective after 15 min. at 56° C.; 3 were innocuous after 30 min., and of the remainder, 3 retained their infectivity after such heating for 180 min.

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Definitive data on the effect of light are limited. Exposure of infected organ suspensions to direct sunlight for 1 hour had no effect on the virus (Iyer, 1943). Exposure of virus-bearing fluid to short ultraviolet rays (1600 to 1800 Å) inactivated the strains studied in 0.8 to 1.08 seconds (Brandly *et al.*, 1946a).

* The literature on Newcastle disease has become so voluminous that references in this paper have been limited largely to reviews and to the selected reports that are available to the authors.

The tenacity of Newcastle disease virus under poultry house environments is well demonstrated by Dobson's experience (1939) One-day-old chicks became infected after being placed in dirty' battery brooders 7 weeks after removal of Newcastle disease infected chickens Chickens placed in an un cleaned pen 2 weeks after the removal of fowls sick with Newcastle disease developed specific viral antibodies without prior overt signs (Jungherr 1948) Newcastle disease virus remained viable for at least 6 months in the bone marrow and muscle tissue of fowl carcasses held under trade chilling conditions (Doyle 1933) The skins of both dressed and plucked infected carcasses of market poultry stored at 24 to 36° F (−5 to 2 C) retained their infectivity for at least 60 days (Gordon Reid and Asplin 1948) Similar studies with carcasses and tissues of artificially infected fowl showed that Newcastle disease virus survived in them for 98 to 160 days (Asplin, 1949) According to Olesiuk (1951) strain 9251 survived in fresh eggs under certain storage conditions for the following periods incubator 126 days normal room 235 days hen house 255 days refrigerator 538 days (last test) The virus remained viable on chick down for the following numbers of days incubator 87 normal room 192 hen house 255 (last test) refrigerator 538 freezer chest 538 (last test)

Substantial resistance of Newcastle disease virus to destruction by variou chemical agents commonly employed for disinfection including the phenolic compounds and formalin as suggested by the work of Doyle (1927) and Farinas (1930) has been verified by more critical later studies (Tilley and Anderson 1947 Cunningham 1948 Moses 1948 Beamer and Prier 1950) Triethylene glycol vapors in saturated concentrations at 23° C and 50 per cent relative humidity were found to be quickly lethal to Newcastle disease virus aerosols (Krugman and Swerdlow 1949) yet in trials in a large broiler plant (Ellis Brandly and Hanson 1952) and with artificial infection among chicks (University of Illinois unpublished data) triethylene glycol vapors failed to prevent the spread of even benign Newcastle disease infection

The properties of virulence and pathogenicity which are inseparable in the complex interaction between the host and its viral parasite are paramount in assessing the significance and potential of Newcastle disease Certain strains of Newcastle disease virus are lethal for chickens embryonating avian eggs and sometimes for various other species whereas other strains which may be variants of the lethal ones are of low or indifferent pathogenicity Prior or concurrent devitalizing factors including environment and other diseases may increase the susceptibility of the host and later the severity of Newcastle disease (Francis and Kish 1952 Sinha Hanson and Brandly 1957) This may be seen in the markedly adverse reactions resulting from complications of vaccinal infection (Brandly and Winslow 1947 McDougle 1948 Binns Nelsen and Miner 1949) Age as a susceptibility factor is well recognized in mammals (Upton *et al* 1955) as well as in poultry (Brandly 1950 and elsewhere) Nevertheless deviations from the highly fatal fulminating disease that identified the initial and early outbreaks of Newcastle disease (Doyle 1927 Kraneveld 1926) were not recognized until several years after the first appearance of Newcastle disease in the United States (Brandly 1953, Beach

1944 Beaudette and Hudson 1956) Wild strains of low pathogenicity identified later include the low form from England (Anon 1948) the English F strain (Asplin 1947) the B_1 or Blacksburg strain (Hitchner Reising and Van Roekel 1951) one associated with an outbreak in Germany (*Hilbrich* 1952) and the Roakin strain (Beaudette Bivins and Miller 1949)

Markedly emphasizing the great variability in pathogenicity and other properties of Newcastle disease virus were the differences found by Hanson (1949) among various strains of Newcastle disease virus Among 18 strains studied pathogenicity varied from 0 to 10^9 LD_{50} doses per 0 5 ml of infective allanto-amniotic fluid and the tropisms varied from low to high degree of pneumal visceral and neural Aside from its capacity to invade and involve the central nervous system of the postnatal fowl and mammal Newcastle disease virus was shown to produce developmental defects in chicken embryos following inoculation of the embryonating egg (Williamson Blattner and Robertson 1953)

Differences or changes in tissue tropisms among strains of Newcastle disease virus can account for variations in such properties as invasiveness of the virus and in the signs course and pathological changes resulting from Newcastle disease infection (Jungherr and Terrell 1946) Furthermore evidence was advanced to indicate that aside from individual and host factors differences in pathogenicity and infective dosage route of infection and particularly degrees of partial immunity prior to infection act as principal modifiers of pathologic expressions of the disease

The established tenacity of Newcastle disease virus obviously contributes significantly to its perpetuation and spread In addition its pneumotropic affinities favor air borne liberation especially via infective droplets and particles from the respiratory system Infection by way of the alimentary tract with eggs and through coitus together with elimination via all body orifices and from the integument during life of fowl afflicted with Newcastle disease further assures extensive dissemination of the virus With ready spread of often concentrated infection there is provided opportunity which history indicates Newcastle disease virus has utilized efficiently for variation and mutation The latter characteristic reinforced by the multiple tissue tropisms of Newcastle disease virus can account in part at least for its recognized adaptability to host species other than fowl

Continued frequent contact via respiratory conjunctival and other routes *may facilitate development of Newcastle disease to person to person* contagions and among individuals of other mammalian species (Brandly 1950 1952 Quinn *et al* 1952 Kyle MacFadden and Gunderson 1952) Evans (1955) pointed out that emergence of variants of Newcastle disease virus caused the respiratory form of the disease in man and may result in man to-man transmission of the infection in the future

World Wide Dissemination of the Virus

The recorded history of Newcastle disease emphasizes the consequences of lack of sanitary regulation and control of the disease upon its further dissemina

tion from its point of origin in Indonesia, where it was described in 1926 as a previously unknown fulminating malady In England Newcastle disease was summarily eradicated by slaughter and terminal disinfection following its first outbreak there in the same year (Doyle, 1927) During the decade following recognition of what is now generally called Newcastle disease the malady was reported from India the Philippines Japan Korea Australia Ceylon and Kenya Subsequently it was recognized in Syria Palestine, and the Middle Congo and during World War II it spread to Europe by way of Sicily and continental Italy It was first identified in the United States in 1944 (Brandly *et al*, 1944 1946 Beach 1944) but it may have found its way there as early as 1935 (Beach, 1944 Beaudette and Hudson 1956) The present world wide diffusion of Newcastle disease was inevitable in the absence of land and sanitary barriers between adjacent areas or across water and air routes of transport Inevitable also is the continued opportunity for Newcastle disease virus to adapt to new animal hosts including man

The identity of Newcastle disease in its initial appearance in these and later foci was seldom recognized with the result that many synonyms including pseudofowl pest *atypische geflugelpest* pseudopoultry plague, avian pest *Ranikhet* avian distemper and pneumoencephalitis have been recorded (Brandly *et al* 1946) Handicapped by lack of definitive laboratory diagnostic procedures and by the differences in clinicopathological features of the disease as it appeared in new areas recognition of the malady and application of control measures were frequently delayed The delay in suspecting and identifying Newcastle disease after it had invaded the United States can be ascribed largely to the previously undescribed features of the disease namely low mortality and predominance of respiratory and nervous signs

The low mortality rate was also responsible in large part for the attitude of the poultry industry of the United States in opposing the accepted principle of eradicating newly introduced exotic maladies as was done with foot and mouth disease Nevertheless the development of mild or subclinical expressions of the disease whether through change in the virus the host or both make it more difficult to detect and especially, to eradicate Newcastle disease being a contagion that is highly diffusible

Source of Infection

A primary source of infection for chickens and one source of infection for man is the chicken in the initial stage of disease Beginning as early as 24 hours before the development of respiratory signs and continuing for several days thereafter diseased birds discharge virus into the air that they exhale (Sinha Hanson and Brandly 1952) The virus has been recovered from small samples of air taken within a few inches of the heads of such birds and it also has been obtained from the air in poultry houses in which a number of diseased birds were present (DeLay *et al* 1948 DeOme *et al* 1948) The respiratory discharges contribute almost all of the virus that exists in the form of the natural aerosol to which poultrymen are exposed Fecal discharges except in the instance of infection with one of a few exotic strains are relatively free of virus

Artificial aerosols are also a considerable hazard to man Fluid virus is sprayed and nebulized as a means of vaccinating chickens (Johnson and Cross 1951 Hitchner and Reising 1952) Virus dust preparations are used for the same purpose Those who provide vaccinating service to poultrymen and poultrymen who do their own vaccinating are subject to exposure to these aerosols for periods varying from a few minutes to several hours and at intervals as frequent as 5 times weekly and as infrequent as once or twice a year Strains of low pathogenicity for chickens are used for vaccines but it has not been shown that these strains are less hazardous for man (Quinn Hanson and Brown 1952)

The laboratories in which the vaccines are produced and in which the virus is studied provide other and important opportunities for exposure of man to viral aerosols The virus is isolated in embryonating chicken eggs for diagnosis and is propagated in them for the production of vaccine The shell is broken in harvesting the infective fluids and tissues and the desired materials are removed with a suction device and transferred to mixing containers The virus material is ground centrifuged and lyophilized before packaging Aerosols may be created in transferring grinding or centrifuging operations (Stein and Rogers 1950) When lyophilization is done in a bulk drum the dried virus is ground to a fine powder and distributed in measured quantities to the final vial This process exposes the operator to a high and continuous virus aerosol (Evans 1954) *Such individuals usually have good serologic titers*

Even in the incubative stages of the disease the carcass of the chicken contains some virus (Hofstad 1951) The concentration is the highest in the viscera particularly in the lungs and spleen although detectable quantities may be found in the blood and even in the muscle Individuals working in eviscerating plants and in diagnostic laboratories are exposed when handling such materials Virus may be carried to the eyes or nose by the spattering of blood and tissue substances or it may be transferred to the eye by contaminated hands *The edible* carcass should under usual conditions of marketing be quite free of virus and an instance of human infection by the ingestion or handling of dressed poultry is unknown

Diseased carcasses and particularly viscera may be a source of infection to chickens (Hess 1951 Lloyd 1952) It is possible that scavengers among rodents and carnivores may distribute the disease by eating dead chickens and then contaminating food supplies with their excreta (Polci and Silvagni 1955 Bolin 1948 Walker and McKercher 1954)

Since a viremia occurs in Newcastle disease of chickens research workers have suspected that biting arthropods may play some role in the epizootiology of the disease Little support has been found for this idea Lice and mites do become engorged with the virus and it has been detected in them by grinding and injecting material into eggs but no virus has been transferred by the bites of arthropods (Bolin 1948 Hofstad 1949) Furthermore instances in which Diptera were reported to transmit the disease have not been confirmed (Picard 1934) It is doubtful that man is exposed to the virus by the agency of arthropods

Disease in Man

Burnet (1943) was the first to report the pathogenicity of Newcastle disease for man. An individual harvesting virus from an embryonated egg in his laboratory was infected by material that splashed into an eye. Conjunctivitis developed within 24 hours, and the virus was isolated from washings obtained from the affected eye. Most of the subsequent cases in man have followed a similar course, with a mild to severe conjunctivitis and spontaneous recovery.

More than 100 cases have been observed since this initial one, and numerous reports have appeared in the literature (TABLE 1). The incubation period is usually 1 or 2 days, but it may occasionally be 3 or 4 days. The initial signs of infection of the eye are irritation and lacrimation. The eye appears red from the injection and swelling of the subconjunctival tissues. The inflammation may become more severe in the next day or two. The lid may also become edematous, and the preauricular lymph node may become palpable. Nelson *et al.* (1952), who observed 40 cases in man, reported that the preauricular adenitis developed on the infected side of 50 per cent of the patients. They found only one eye infected in almost all of the cases, and observed that constitutional symptoms were rare. The conjunctivitis lasts for 3 or 4 days in most patients, but courses of 7 to 8 days and even of 21 days have been reported.

A description of cytoplasmic inclusions presumed to be pathognomonic in cells obtained from conjunctival scrapings on the third, fourth, and fifth days (Keeney and Hunter, 1950) has not been confirmed by other observers (Orlandella, 1955). Blood changes do not appear to be consistent. The cornea, the extraocular movements, and the visual field remain normal, and the pupil reacts normally. Recovery is usually uneventful and complete within a week or two. Occasionally, subsequent impairment of visual accommodations has been reported.

Virus has been isolated from conjunctival washings obtained on the second, third, fourth, and fifth days after the onset of symptoms. In some instances, virus has been isolated from other secretions and tissues. Antibodies have been detected as early as 7 days after onset but, more frequently, 14 to 21 days later. The immunological response is variable. Some individuals from whom the virus has been isolated do not develop hemagglutination inhibition or serum neutralizing antibodies. In others, hemagglutination titers of 1:16 to 1:160 and serum neutralizing titers of 1:100 to 1:100,000 have been reported. Among employees of a chicken processing plant, Nelson *et al.* (1952) found that 64 per cent of the exposed individuals not reporting conjunctivitis had high neutralizing titers, and only 25 per cent of those reporting conjunctivitis had high neutralizing titers. None of the unexposed controls had significant titers.

A generalized infection of man has been reported by numerous observers (TABLE 1). In some instances it has been a mild influenza-like infection characterized by rise in body temperature of 2 or 3° F, with chills and pharyngitis (Mitchell and Walker, 1951). Conjunctivitis may or may not be associated with this syndrome (Quinn *et al.*, 1952). The infection lasts 3 to 4 days. The exposure of most individuals in whom the generalized infection has devel

TABLE I
NEWCASTLE DISEASE OF MAN

		Year	Notes
Burnet F M	Med J Australia 2 313	1943	first isolation
Anderson S G	Med J Australia 2 371	1946	
Shimkin N I	Brit J Ophthalmol 30 260	1946	
Yatom J	Harefuah 30 47	1946	17 clinical cases
Kadrat M	Ophthalmol 113 106	1947	
Howitt B F, J K Bishop & R F Kissling	Am J Public Health 38 1263	1948	(see Howitt 1950)
Hurt L M	Los Angeles [illegible] D Rept	1948	
Kujumgiev J	[illegible] 3 76	1948	
Pomeroy B S & R Fenstermacher	U S Egg Poultry 54 19	1949	
Bawell M B, M LeGier, T Merrill, W Schofield & G O Brown	J Lab Clin Med 34 1591	1949	isolation from lung
Berke Z & S B Golem	Türk Ijien Tecrübi Biol Dergisi 9 1	1949	
Freymann M W & F B Bang	Bull Johns Hopkins Hosp 84 409	1949	
Ingalls W L & A Mahoney	Am J Public Health 39 737	1949	
McGough F F	Ohio State Med J 45 25	1949	later invalidated
Sinkovits J	Kisérletes Orvostudomány 1 134	1949	
Howitt B F	J Immun 64 73	1950	(see Howitt 1948)
Jacotot H, A Vallee & A Le Priol	Bull acad natl méd Paris 134 106	1950	
Keeney A H & M C Hunter	A M A Arch Ophthalmol 44 573	1950	
Lépine P, P Atanasiu & H G Gabreau	Rev méd vét parasitol 6 1054	1950	
Sheehan G A	J Am Vet Med Assoc 66 219	1950	
Evans A S	J Immunol 67 529	1951	serologic survey
Gustafson D P & H E Moses	J Am Vet Med Assoc 118 1	1951	
Hunter M C, A H Keeney & M M Sigel	J Infectious Diseases 88 272	1951	(see Keeney 1950)
Latte B	Bull Ocul 30 553	1951	
Melnick J L & N Ledinko	Am J Hyg 54 354	1951	serologic survey
Mitchell C A & R V L Walker	Can J Comp Med Vet Sci 15 226	1951	influenza like disease
Dis A & A Te	Bol inst invest vet 4 644	1952	case of parotiditis
Esquerm A & J Dat[illegible]	Ann inst Pasteur 83 407	1952	clinical survey
Liepman O	Am J Ophthalmol 35 104	1952	
Moulten S E & J Clark	A M A Arch Internal Med 89 2 0	1952	case of anemia
Nelson C B, B S Pomeroy, K Schrall, W E Parks & R J Lindeman	Am J Public Health 42 672	1952	outbreak in a processing plant

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HOWITT B F L. K. BISHOP & R E. KISLING	Am J Public Health 38 1263	1948	(see Howitt 1950
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KUJUMGIEV J	Zooprofilassi 3 6	1948	
POMEROY B S & R FENSTERMACHER	U S Egg Poultry 64 19	1948	
BAWELL M B M LEGIER F MURRAY W SCHOFIELD & G O BROWN	J Lab Clin Med. 34 1581	1949	isolation from lung
BERKE Z. & S B GOLEM	Turk Ij en Tecrubi Biol. Dergisi 9 1	1949	
FREYMANN M W & F B BANG	Bull Johns Hopkins Hosp 84 409	1949	
INGALLS W L. & A. MAHONEY	Am J Public Health 39 3	1949	
McGOUGH I F	Ohio State Med J 45 25	1949	later invalidated
SINKOVITS J	Kiserletes Orvostudomány 1 134	1949	
HOWITT B F	J Immunol 64 3	1950	(see Howitt 1948)
JACOTOT H., A VALLEE & A LE PRIOL	*Bull acad natl méd Paris 134* 106	1950	
KEENEY A. H & M C HUNTER	A M A. Arch. Ophthalmol. 44 5 3	1950	
LÉPINE P., P ATANASIU & H G GABREAC	Rev med vet y parasitol 8 1034	1950	
SHEERAN G. A	J Am Vet. Med Assoc 66 719	1950	
EVANS A. S	J Immunol 67 579	1951	serologic survey
GUSTAFSON D P & H E. MOSES	J Am. Vet. Med Assoc. 118 1	1951	
HUNTER M C A. H KEENEY & M M SIGE	J Infectious Diseases 88 2 ?	1951	(see Keeney 1950)
LATTE B	Bull. Ocul 30 553	1951	
MELNICK J L. & N LEDINKO	Am J Hyg 54 354	19 1	serologic survey
MITCHELL C A. & R. V L. WALKER	Can J Comp Med Vet Sci 15 226	1951	influenza like disease
DINO A. & A FEGO	Bol inst. invest. vet 4 644	19 2	case of parotiditis
EYQUEM A & J DAUSSET	Ann inst. Pasteur 83 407	1952	clinical survey
LIPPMAN O	Am J Ophthalmol 35 104	19 ?	
MCKELTEN S E & E CLARK.	A. M A. Arch. Internal Med 89 2 0	1952	case of anemia
NELSON C B B S POMEROY K SCHRALL, W E PARKS & R J LINDEMAN	Am J Public Health 42 6 2	1952	outbreak in a processing plant

TABLE 1—*Continued*

		Year	Notes
NEGRI R A D'AMORE & L RAVAIOLI	Atti soc ital sci vet 6 562	1952	
QUINN R W R P HANSON J W BROWN & C A BRANDLY	J Lab Clin Med 40 736	1952	isolation from blood and urine
SLONIM D & V STRANUKOVA	Bull Hyg 27 708	1952	
TOPOLNIK F & A H BEGANOVIC	Vet Arch 21 572	1952	
WENNER H A M H JENSON & A MONLEY	J Immunol 68 343	1952	
BIELING R & O SIEGMANN	Deut med Wochschr 78 1037	1953	
MITCHELL C A	Proc Book Am Vet Med Assoc 432	1953	encephalitis like disease
MOOLTEN S E F CLARK B F GLASSER E KATZ & B S MILLER	Am J Med 14 294	1953	(see Moolten 1952)
NEGRI R A D'AMORE & L RAVAIOLI	Rend ist super sanità 16 183	1953	
PASCUAL	J Am Med Assoc 151 567	1953	case of jejunitis
RODOT M	Thesis Paris (Alfort)	1953	
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SIEGERT R H G HAUSMANN & E MANNWEILER	Klin Wochschr 32 8	1954	
AMADEO G	Gaz Vet 3 17	1955	
ANON	Zootria Chile 4 75	1955	
EVANS A S	Am J Public Health 45 742	1955	
FRANCO A S	*Bol inform S y V A 7 3*	*1955*	
JACOTOT H A VALLÉE & A LE PRIOL	Ann inst Pasteur 88 111	1955	
ORLANDELLA V	Nuova vet 31 206	1955	
VETTERLEIN W	Deut Gesundheitsw 10 1327	1955	

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to invade the central nervous system through the blood system or even along the peripheral nerves While there is considerable evidence that systemic infection of man sometimes occurs some of the reports are open to criticism (Morgan 1952)

The immunology of Newcastle disease in man is perhaps the most controversial aspect of the disease In chickens the virus induces the production of antibodies that inhibit the hemagglutinin of the virus and neutralize its infectivity for a susceptible host Hemagglutination inhibition titers of 1 1280 and serum neutralizing titers of 1 1 000 000 are not uncommon and they develop within 14 to 21 days after the initiation of infection Birds with circulating antibodies are immune to challenge Specific antibodies detectable by hemagglutination inhibition serum neutralization and complement fixation tests may be produced in rabbits cattle and horses While some serologic differences exist among strains of the virus all strains cross react to a considerable degree with antibodies produced to heterologous strains

In man infection may or may not result in the production of antibody detectable by hemagglutination inhibition and serum neutralization tests Man may or may not be refractory to a second exposure to the virus (Nelson *et al* 1957) Reinfection has been reported within 3 or 4 months of the initial infection Further complicating the problem are the nonspecific neutralizing substances that may be present in the serum of man Howitt (1950) has described a heat labile neutralizing substance found in many human sera This substance is destroyed at 56 C in 30 minutes and it is also irreversibly fixed to filter paper upon which the serum has been dried A second heat stable substance that neutralizes Newcastle disease virus and inhibits the hemagglutinin is found in some sera It is associated with the presence of neutralizing antibodies for mumps virus Wenner Jensen and Monley (1952) contend that the substance may be partially inactivated by heating at 56 C for 30 minutes and that accordingly it is not specific and is not an antibody shared by mumps and Newcastle disease Evans (1955) utilized cross adsorption procedures and found that in human sera that neutralized both mumps and Newcastle disease virus there were two possibilities (1) Newcastle disease virus alone could adsorb what was Newcastle disease virus antibody and (2) either the Newcastle disease virus or mumps virus could adsorb a Newcastle disease neutralizing substance It has been shown repeatedly that Newcastle disease antibody will not neutralize mumps virus and that man or animals immunized against a single strain of mumps virus fail to produce substances capable of neutralizing Newcastle disease virus Nevertheless such cross reactions are common during convalescense from naturally acquired mumps infection Evans (1955) interprets this to mean that more than one strain of mumps virus exists and that at least one of these has an antigen that is common or related to those present in some strains of Newcastle disease virus One factor that had contributed to the controversy is the lack of uniformity in the procedure by which the neutralization test is conducted Investigators of the disease in birds have found that serial dilutions of virus and undiluted serum gave a sensitive and reproducible result while the reverse

TABLE 1—*Continued*

		Year	Notes
NEGRI R A D AMORE & L RAVAIOLI	Atti soc ital sci vet 6 562	1952	
QUINN R W R I HANSON J W BROWN & C A BRANDLY	J Lab Clin Med 40 736	1952	isolation from blood and urine
SLONIM D & V STRANTKOVA	Bull Hyg 27 708	1952	
TOPOLNIK E & A H BEGANOVIC	Vet Arch 21 572	1952	
WENNER H A M H JENSON & A MONLEY	J Immunol 68 343	1952	
BIELING R & O SIEGMANN	Deut med Wochschr 78 1037	1953	
MITCHELL C A	Proc Book Am Vet Med Assoc 432	1953	encephalitis like disease
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ANON	Zootria Chile 4 25	1955	
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lishments on which several million chickens are raised each year to small flocks of less than 100 that are kept to supply the farmer s wife with egg money To this population of 7 or 8 million people who feed and water chickens and clean out chicken houses must be added the several hundred thousand people engaged in the processing of poultry and a few thousand more employed in occupations in which the virus is handled It should not be inferred that all of these people are at equal risk almost all of the infections that have been reported have come from the two smaller groups which however are under much closer medical observation than the larger group Every recorded instance of human infection has been an initial case the transmission being from bird to man and not from man to man

The greatest importance of Newcastle disease is its cost to the consumer as reflected in higher prices for poultry products and in poorer quality of the product The disease may or may not produce a high mortality in chickens depending on the strain of the virus the age and condition of the individual bird and certain environmental factors A strain of virus isolated in Texas a few years ago killed 9 out of 10 chickens irrespective of age (Boney 1951) Other strains have killed only 1 of 100 chicks a day old

A fallacy believed by many consumers is that the death of the chicken is the important loss to the producer and that if the chicken survives the disease the producer does not suffer Actually when the disease does not kill it usually affects the chicken by interrupting its growth and egg production and some times by damaging the reproductive tract so that the hen lays eggs with poor shells and an inferior albumen (Biswall 1954 Parnell 1950 Knox 1950)

Interruption of growth for one week would mean added feed cost of 10 cents per bird Such an increase could wipe out the margin of profit in broiler pro duction and mean a net loss to the producer Another factor adding to his loss is the increase in the number of culls that must be rejected from the dressing line Interruption of egg production for 2 to 4 weeks is equally disastrous To this is added the production of eggs that are downgraded and consequently sell for a lower price All of these things are reflected in the amount of meat and eggs that are available to the consumer in the United States at a reasonable cost

The control of animal disease in the United States has progressed success fully to complete eradication of but one poultry disease namely fowl plague To date there has been no serious discussion of an eradication program for Newcastle disease or even of an organized control program When the disease was first recognized in the United States an attempt was made to stay its spread by quarantine but this approach was abandoned as vaccines came into use The disease is now found in all sections of the country in which poultry *is raised The incidence varies from year to year but there* is no evidence of retrenchment Individual poultrymen attempt to protect their flocks against the infection by vaccination if the risk warrants it This decision is based on the experience of the previous seasons and the current reports of the disease in the neighborhood Vaccination adds about one or one and one half cents to the cost of producing a broiler On a national basis this would amount to more

procedure, serial dilution of the serum with a constant amount of virus was unsatisfactory Evans (1955) demonstrated that the same situation holds for human sera in a neutralization test for Newcastle disease virus While more sera are found to contain antibody and while the titers are higher the use of heat inactivated undiluted sera in neutralization tests for Newcastle disease virus does not in all instances result in the detection of antibody following exposure

There appears to be little question that where an opportunity for contact with Newcastle disease virus exists a considerable proportion of the humans so exposed will develop a transient conjunctivitis Systemic infection of man may sometimes occur The history of Newcastle disease in man with its conflicting reports gives ample reason for a questioning attitude toward systemic infection of man Serology while sometimes helpful is undependable

Isolation of the virus is a difficult procedure for routine diagnosis but it is much more meaningful than are serologic findings Unfortunately isolation also can be misleading for two reasons

First spurious isolation may occur by contamination of the tissue under test with virus already present in the laboratory in which the isolation is being made It is possible but very unlikely that the virus might be present in the eggs used for isolation These problems can be avoided by taking adequate precautions in the selection of procedures and of materials and by demonstrating that the virus can be reisolated from another aliquot of the original material

The second reason for doubt is the frequently inadequate attention given to the possibility of other concurrent infections either bacterial or viral For example the common cold may be acting simultaneously with a specific Newcastle disease infection of the eye to produce what appears to be Newcastle disease conjunctivitis associated with a generalized Newcastle disease infection This objection can be answered only after study of many case histories reveals one or more consistent disease patterns associated with *Newcastle disease infection It is necessary that consideration always be* given to the possibility that more than one clinical pattern of the disease since there are many strains of Newcastle disease virus and since they may be introduced by more than one route Conjunctivitis only may occur when some strains of virus are introduced by splattering while a generalized infection may occur when other strains are introduced as aerosols

A diagnosis of Newcastle disease of man should be dependent upon a history of contact with the virus a short incubation period the development of conjunctivitis or possibly influenzalike symptoms and the isolation of the virus under satisfactory conditions

Newcastle disease of man has been a minor occupational disease limited to the people employed in the production of chickens and eggs and in processing them for the market and to those concerned with the diagnosis of the disease or the production of vaccine The population at risk is rather sizable as there are approximately 3 418 000 farms in the United States on which poultry are kept (1954 United States Census Report) This includes huge poultry estab

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than 4 million dollars exacted as a tribute to Newcastle disease in 1956 a year in which 474 million broilers were marketed Newcastle disease will probably remain a serious problem for some time to come The trend to larger and larger plants for the production of eggs and poultry and the increased concentration of these plants in selected areas increase the problem of air borne infection This increases the need for periodic vaccination a requirement that is being met by the use of aerosol products requiring less labor in their administration than did the older methods The direction is toward fewer men in the production of poultry products but these individuals are exposed to aerosols of Newcastle disease virus more frequently and over prolonged periods

Newcastle disease has been reported in many species of wild and domestic birds (Blaxland 1951 Gillespie Kassell and Fabricant 1950 Gustafson and Moses 1953) The virus may become better adapted to sparrows or pigeons, and these birds could then serve as sources of infection to man Whether such a virus would be more or less pathogenic for man is pure speculation Burnet suggested in 1942 that Newcastle disease virus might be a potential human pathogen still in its avian form, since eventually it might, through selection afforded by frequent exposure develop a mammalian form capable of perpetuation in man Burnet has postulated that such a development from an avian form to a mammalian form was the origin of influenza

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TABLE 1
TOTAL REPORTED CASES IN THE UNITED STATES 1946 TO 1955

	Typhoid	Paratyphoid and other salmonelloses
1946	3768	723
1947	3075	951
1948	2840	882
1949	2795	1243
1950	2481	1233
1951	2128	1733
1952	2341	2596
1953	2252	3946
1954	2169	5375
1955	1704	5447
Total	23 352	24 129

TABLE 2
FOOD POISONING IN THE UNITED STATES BY OUTBREAKS

	Salmonellae	Staphylococci	Others	Total
1953	21	81	84	186
1954	26	100	126	252
1955	16	102	77	195
Total	63	283	287	633

Including 236 outbreaks of gastroenteritis of undetermined etiology

TABLE 3
FOOD POISONING IN ENGLAND AND WALES 1950 TO 1955 BY INCIDENTS

	Salmonellae	Staphylococci	Others	Total
1950	2071	87	1816	3979
1951	1668	65	1614	3347
1952	2098	8	1339	3519
1953	3114	137	2031	5277
1954	3508	127	2381	6016
1955	5269	138	3554	8961
Total	17 678	676	12 795	31 099

Including 12 464 incidents of undetermined etiology

the Ministry of Health of Great Britain[6,10] for the years 1950 to 1955 inclusive (TABLE 3) These figures represent incidents or episodes which are made up of outbreaks involving more than one family family outbreaks and sporadic cases Food poisoning in all categories increased during the period illustrated and in each year salmonellosis was responsible for approximately 50 per cent or more of the incidents In contrast staphylococcal poisoning comprised only 2 per cent of the incidents If one considers general outbreaks family out

SALMONELLOSIS OBSERVATIONS ON INCIDENCE AND CONTROL

By P R Edwards
Communicable Disease Center Public Health Service Atlanta Ga

In any discussion of the prevention of the transmission of a disease from animals to man it is desirable first to establish the incidence of the condition in man and in the animal hosts from which it was transmitted In the case of salmonellosis it is impossible to determine accurately the incidence of the condition either in man or animals No systematic method of reporting salmonellosis of animals has been established and one must rely only on summaries of isolation and identification of causative agents that are dispersed throughout the medical and veterinary literature to estimate the frequency with which the bacteria occur among the lower animals

The difficulty of establishing the incidence of salmonellosis in man has been dwelt upon at length by Meyer[1] in his excellent monograph on food poisoning Here it was pointed out that the summaries published annually by the National Office of Vital Statistics Public Health Service Bethesda, Md were based upon woefully inadequate reporting from a minority of the states and that in most instances the etiological agents of food borne infections were not determined accurately In spite of these deficiencies one must rely upon these summaries for any estimate of the prevalence of *Salmonella* infections in the United States In TABLE 1 are listed the reported cases of typhoid fever and other salmonelloses for the ten year period 1946 to 1955 It is apparent that while typhoid fever gradually declined during this period paratyphoid fever and other salmonelloses increased evenfold MacCready Reardon and Saphra[2] observed a fourfold increase in incidence in Massachusetts from 1940 to 1955 It is not possible to say what part of this rise represents an actual increase in the number of cases and what portion is due to more accurate diagnosis and better reporting Probably each factor has some influence on the figures which inadequate though they are demonstrate a growing awareness of the problem on the part of state health agencies

If one turns to outbreaks of salmonellosis and other food poisonings the figures included in TABLE 2 may be extracted from the data compiled by Dauer and Sylvester[3-5] Outbreaks of bacillary dysentery have been excluded for obvious reasons Only 10 per cent of the recorded outbreaks were attributed to salmonellosis and these included but 18 per cent of the cases of *Salmonella* infection reported for the corresponding years On the contrary outbreaks of staphylococcal poisoning reported were 4 5 times as numerous as outbreaks of salmonellosis It is noteworthy that insofar as can be ascertained from the publications salmonellae were isolated in connection with each outbreak of salmonellosis reported but in the 202 staphylococcal outbreaks reported for 1954 and 1955 staphylococci were isolated from food food handlers or patients in only 20 per cent of the episodes

The data described above may be contrasted with the figures compiled by

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the Ministry of Health of Great Britain[9,10] for the years 1950 to 1955 inclusive (TABLE 3). These figures represent incidents or episodes which are made up of outbreaks involving more than one family, family outbreaks and sporadic cases. Food poisoning in all categories increased during the period illustrated and in each year salmonellosis was responsible for approximately 50 per cent or more of the incidents. In contrast, staphylococcal poisoning comprised only 2 per cent of the incidents. If one considers general outbreaks, family out

TABLE 4
FOOD POISONING IN ENGLAND AND WALES, 1950 TO 1955, BY INCIDENTS

	General outbreaks	Family outbreaks	Sporadic cases	Total incidents
Salmonella	920	1986	14 772	17 678
Staphylococcus	303	182	141	676
Other	1641	687	10 467	12 795
Total	2864	2855	25 380	31 099

TABLE 5
OUTBREAKS PER 100 000 POPULATION, 1953–1955

	England and Wales	United States
Salmonella	1.17	0.042
Staphylococcus	0.4	0.18

breaks, and sporadic cases separately for the same period, the data in TABLE 4 are obtained. The proportion of staphylococcal incidents is greater among the general outbreaks than among family outbreaks and sporadic cases, but even when distinct outbreaks only are considered, *Salmonella* infections outnumber staphylococcal poisonings by 3 to 1. Furthermore, it is evident that the great majority of the former occurred as sporadic cases, a fact borne out by our own experience.

Objection has been taken to the British data on the ground that workers in England were prone to ignore staphylococcal poisonings and that only in recent years have British bacteriologists taken full cognizance of the disease.[1] It is felt that these objections are based only upon the preponderance of *Salmonella* infections in the British reports. The data in TABLE 5 are presented in confirmation of this view. From these data it is evident that more than twice as many outbreaks of staphylococcal poisoning per capita were reported in 1953, 1954, and 1955 in England and Wales as in the United States. For the same period, the per capita reports of outbreaks of salmonellosis were 28 times as numerous as those in the United States. It seems very unlikely to me that the incidence of the two diseases in the two countries actually could be so different, particularly in view of the report of a committee of the Medical Research Council, London, England,[11] that importation of egg powder from the United States added greatly to the problem of salmonellosis in Great Britain. Rather, it would seem that reporting of salmonellosis in England and Wales is much more complete than in the United States, and that only a small fraction of the outbreaks in the latter country are diagnosed and reported. Furthermore, it must be remembered that sporadic cases and family outbreaks greatly outnumber the dramatic occurrences that draw the attention of the press and the public as well as of the agencies that deal with public health. It can be concluded only that endemic salmonellosis constitutes a considerable problem in the United States, as it does in other countries.

That salmonellae are widely dispersed and occur frequently among the various species of lower animals is evident from numerous reports in the literature This statement applies not only to the host adapted types (the so-called primary salmonellosis of many species) but to the nonhost adapted types as well Improved methods of isolation and identification have made the prevalence of the bacteria increasingly apparent within recent years Not only do some types occur both in animals and man[1, 14] but there is a distinct correlation between the presence of the organisms in lower animals and in the human population For instance Ranta and Dolman[17] noted the high incidence of *S thompson* in man in western Canada and Gibbons and Moore[18] isolated many cultures of this type from egg powder produced in the same locality Atkinson *et al*[19, 20] called attention to the frequent occurrence of *S bovis morbificans* in animals and man in Australia Neither of these types was found frequently in material from similar sources in the United States at the time the observations were made Likewise Kauffmann[21] and Van Oye[22] have noted the presence of a number of rare and exotic serotypes in man and various animal species in the Belgian Congo and Stewart and DeCapito[3] noted the similarity of types isolated from man and animals in the southeastern United States Furthermore dramatic increases in the incidence in both man and animals of serotypes previously seen very rarely or not at all in a given region often are observed In the United States *S heidelberg* and *S blockley* are excellent examples Such a list of occurrences could be extended greatly and the observations imply that there is a direct correlation between salmonellosis of animals and man in any given locality although the methods whereby the organisms are transmitted from animals to man and vice versa may vary from one region to another depending upon sanitary facilities and habits of the populations The literature dealing with the presence of salmonellae in animals is so voluminous that it is possible only to mention representative observations that deal with the presence of the bacteria in animals used widely as food and in animals which in one way or another come into close contact with man

Ruminants In many parts of the world *Salmonella dublin* is endemic in cattle and often has been transmitted to man through infected carcasses and milk Bartel[24] Henning[25, 6] Savage[27] Field[5] and McCall[9] have reviewed the literature pertaining to the transmission of *S dublin* infection from cattle to man Murdock and Gordon[40] noted the presence of *S dublin* in the feces of 24 5 per cent of apparently healthy slaughter cattle from Eire indicating the extent of the problem in certain areas It is known that many healthy cattle are either temporary or permanent carriers of the bacteria that large numbers of the bacteria are excreted in the feces and that mastitis occurs occasionally in infected cows resulting in the excretion of very large numbers of the organisms in the milk Not only is *S dublin* found in cattle but other *Salmonella* types also occur among them Edwards Bruner and Moran[1] Collard and Sen[2] and Seeliger[15] noted the occurrence of a variety of salmonellae in cattle and Norton and Armstrong[32] described a milk borne outbreak of *S typhimurium* infection due to the presence of an infected cow in a dairy herd

Bartel[4] and Lutje[33] noted the frequent occurrence of *S typhimurium* among cultures from adult slaughter cattle As regards other ruminants it is known that sheep frequently are infected with *S typhimurium* and a variety of other types[12, 34-37]

Swine For some time swine have been known to carry salmonellae and the older European literature abounds in descriptions of mass outbreaks of *S choleraesuis* infections in man due to the consumption of infected pork However it was not until Hormaeche and Salsamendi[38, 39] systematically examined the feces and lymph glands of apparently normal hogs that the number and variety of the bacteria in swine were realized fully These observations were amply confirmed by many workers[40-43] Galton *et al*[41] noted that isolations from swine in abattoirs were much more frequent than from animals on the farm and mentioned the importance of the sales yard the holding lot and the abattoir in the spread of the organisms

Fowl When one considers the extremely large population of domestic fowl and the frequency with which salmonellae are isolated from them[1, 44, 45] birds probably constitute the largest single reservoir of these bacteria among animals The isolation of salmonellae by Perelli Minetti *et al*[46] from 41 4 per cent of all turkeys presented for autopsy in California well illustrates the extent of the problem of salmonellosis in domestic fowl From the results of a number of investigators[11, 18, 47, 52] it is evident that the bacteria frequently are present in egg products Salmonellae may gain entrance to eggs either as a result of ovarian infection or by penetration of the eggshell The conditions under which penetration of the eggshell takes place have been studied by Stokes Osborne and Bayne[53] who found that penetration did not occur in eggs stored at 10 C or below The frequent occurrence of the bacteria in processed eggs was attributed by Solowey *et al*[54] largely to contamination of eggshells These investigators emphasized the desirability of using only clean eggs in processing plants As long as cracked and soiled eggs that cannot be marketed as shell eggs are incorporated into dried and frozen egg products one can expect a high incidence of salmonellae in those products

Other animals Dogs and cats have been incriminated as carriers of salmonellae[55, 61] In some instances direct transmission of the infections from dogs to man has been observed Rodents long have been known to carry *Salmonella enteritidis* and *S typhimurium* and for many years it was thought that rats and mice carried these types exclusively It now is known that wild rats may carry a variety of serotypes[6, 65] and that the types that are carried are largely the same as those found in the environment of the animals

Salmonellae occur also in insects and other cold blooded animals but it is not clear whether their occurrence in these animals constitutes a true carrier state or whether it is merely a response to their environment From the data available on insects it would seem that flies long suspected as vectors of enteric infections carry the organisms both on the exterior of their bodies and in their intestinal tracts but only for a short period after exposure[68, 69] From the results obtained by Watt and Lindsay[70] in fly control studies flies are of less importance in the transmission of salmonellae than of shigellae Cold

blooded animals such as snakes[69] tortoises[71 73] and ticks[79] have been found to carry salmonellae and Thomas[74] observed transmission from a tortoise to a child Salmonellae also have been isolated from fish[80] and from shellfish[81 82] Evidence indicates that waters contaminated by man or animals were responsible for the presence of the organisms in these aquatic animals

Within recent years a number of studies of the presence of salmonellae in sewage polluted waters of rivers and harbors and in wild fowl that inhabit these waters were undertaken in Europe[83 85] It was demonstrated that many river and harbor waters contained numerous salmonellae of a variety of types and that many of these types also were present in gulls terns ducks and other birds that inhabit these waterways Flooding of meadows with these polluted waters led to contamination of the soil and the infection of domestic animals[86] The presence of salmonellae in the irrigation waters of the western United States also has been demonstrated repeatedly[87 88]

Salmonellae in foods It is evident that numerous *Salmonella* types are widely distributed throughout the animal kingdom It is small wonder therefore that the organisms frequently are present in food products of animal origin While it has been known for some time that salmonellae occasionally are present in meats recent investigations have revealed their presence in an unsuspectedly high percentage of them Holtz[86] recovered the bacteria from 3 per cent of 24 442 meat samples and Walzburg[89] did so in 4 per cent of the samples of beef examined Diageler and Kotter[90] found salmonellae in 951 samples of meats and other foods Seeliger[15] recorded the isolation of a number of *Salmonella* types from a variety of food products in Germany From the publications of Cherry Scherago and Weaver[91] and Felsenfeld Young and Yoshimura[92] it was evident that salmonellae found their way into commercial meat products in the United States but it was not until Galton Lowery and Hardy[93] isolated the organisms from 23 per cent of the samples of fresh sausage and from 12 5 per cent of the samples of smoked sausage examined that the extent of the problem was fully apparent The presence of salmonellae in eggs and egg products has been mentioned That these organisms also can be found in dressed poultry is evident from the reports of Cherry Barnes and Edwards[94] Schneider and Gunderson[95] Prowne[96] Galton *et al*[97] and Mackel and Payne (personal communication)

The transmission of salmonellosis from animals to man through contaminated animal food products has been recognized since the days of Gartner[98] and Basenau[99] The literature regarding the transmission of the infections from animals to man is so voluminous that it is possible to mention only a few examples The part played by beef in the production of the disease was reviewed recently by Clarenburg[100] Trub and Schneider[101] noted 418 outbreaks of salmonellosis in Germany in 1951 and 1952 in many of which a variety of animal food products were proved to be vehicles of infection Hauser Treuting and Breiffelh[102] Jones and Symons[78] and Premavesi[10] described outbreaks due to infected sausage and the last named author was able to trace the source of the infection to the swine from which the meat was derived

The role of duck eggs in salmonellosis of man was reviewed by Clarenburg[10]

and by Lerche[106] Clarenburg Vink and Noordhoff[107] and Miller[108] observed cases of salmonellosis due to duck eggs and Yamamoto *et al*[109] incriminated goose eggs in the production of the disease Hen eggs have been found to give rise to human infections[110 11] while frozen whole eggs were incriminated as a vehicle of infection by Newell[113] and Newell Hobbs and Wallace[114] The widespread outbreak of *S montevideo* infection in babies in the United States due to infected dried egg yolk was described by Abramson *et al*[115] Direct transmission from fowl to man by contact infection frequently has been noted[12 45 116 118]

The Role of Food Processing and the Human Carrier in Salmonellosis

While salmonellae obviously are indigenous to many animal food products the role of the human carrier and the processing plant in the contamination of the final product must not be underestimated Clarenburg[100] called attention to the necessity of differentiating between intravital and post mortem contamination of food products Galton *et al*[41] noted that salmonellae were found in carcasses in abattoirs with greater frequency than in swine on farms and they emphasized the role of the abattoir environment and processing procedures in the spread of the organisms Hardy and Galton[119] also emphasized the role of the processing plant in the spread of the bacteria Schonberg[1 0] cited the inadequate facilities extant in many abattoirs and suggested control measures to lessen the contamination of carcass meats Browne[96] and Galton *et al*[97] found salmonellae widely distributed in poultry processing plants The bacteria were found on tables trays mechanical equipment the hands of workers animal carcasses and edible viscera The recovery of *S typhi S paratyphi* 4 and shigellae from market meats by Floyd Baranski and El Gannani[1 1] obviously represents contamination from human sources Outbreaks in which human carriers were responsible for the contamination of meat products have been described[1 125] at length Felsenfeld and Young[1 6] reported that 26 of 56 outbreaks due to nonhost adapted salmonellae the sources of which were established were caused by human carriers The *frequent occurrence of the normal human carrier has often been stressed*[1 15 16 17 18] In a large series of *Salmonella* isolations Edwards Bruner and Moran[1] noted that 29 6 per cent of the cultures from man were isolated from asymptomatic persons while Saphra and Winter[16] reported 15 5 per cent and MacCready Reardon and Saphra[2] 21 9 per cent of cultures from carriers Saphra and Winter[16] estimate the carrier rate in the general population at 0 2 per cent while Savage[129] cites the results obtained by the Public Health Laboratory Service London England in the examination of 9829 persons of whom 0 24 per cent were carriers In the series of cultures described by Galton and Hardy[17] no less than 63 per cent of the cultures of known origin were isolated from asymptomatic carriers Inasmuch as many of these cultures were isolated from food handlers (Galton personal communication) and since Galton and Quan[131] and Galton and Hardy[131] comment upon the frequency with which the organisms are isolated from food handlers it becomes apparent that the carrier state in food handlers occurs much more frequently than in the general

population It might be said that the carrier state is an occupational hazard of those persons who continually handle uncooked meats and carcasses Carriers occur among infants as well as among adults[70, 13, 133] These nonhost adapted salmonellae may persist in man for only a short time or they may persist for very long periods[2, 14, 130, 135] Thomson[134] found that the numbers of salmonellae excreted by asymptomatic carriers varied widely but that the numbers found per gram of feces sometimes exceeded those found in clinical cases Direct transmission from man to man may occur without the intervention of food This phenomenon has been observed frequently in infants in hospital nurseries who may contract infections from other infants or from asymptomatic staff members[133, 135] Infection of babies at parturition by mothers has been observed repeatedly[137, 141] It is well known that both clinical cases and the carrier state in adults may result from contact with clinical cases

Control In any consideration of the control of infections due to nonhost adapted types of salmonellae it is necessary to consider animal reservoirs of infection excretion of the bacteria by human cases convalescents and carriers the presence of the organisms in the environment and the methods whereby they are transmitted among and between man and the lower animals

While there is a large reservoir of salmonellae in the lower animals this situation can be expected to improve gradually Tests for detection of the infections and methods of eradication are being studied in fowl and cattle Progress has been made in the eradication of salmonellae from poultry flocks[142, 144] Lukas and Bradford[145] noted a 50 per cent decrease in the number of turkeys presented for autopsy and a decline in the rate of salmonellosis from 41 per cent to 21 per cent in the cases examined This decline was attributed to an agglutination testing program for *S typhimurium* infection Field[78] and Henning[6] have studied the possibilities of the detection of *S dublin* infection by agglutination tests While the elimination of salmonellosis from flocks and herds cannot be accomplished in the immediate future it is encouraging that animal pathologists particularly poultry pathologists are thinking in terms of eradication of the infections rather than of their control It must be admitted however that this attitude is dictated largely by economic considerations more than by regard for public health In any effort to eradicate salmonellae from domestic animals it is necessary to take into consideration the continuous seeding of the population through infected feedstuffs Galton Harless and Hardy[146] and Griffin[147] have isolated salmonellae from animal feeds Recently fish meal has been incorporated into many feeds and this supplement is known to be a fertile source of salmonellae[15] Rohde[148] isolated ten different *Salmonella* types from one sample of Angola fish meal and Boring[49] repeatedly isolated the bacteria from fish meal produced in the United States Unfortunate incidents have resulted from the addition of egg powder to feeds It will be necessary to eliminate such sources of contamination if *Salmonella* infections are to be eliminated from flocks and herds

As regards dissemination from person to person every effort should be made to prevent the spread of the organisms among the population through the excreta of clinical cases convalescents and carriers As emphasized by

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Grant[135, 150] this aspect of the problem is a joint responsibility of the practicing physician, the health officer, and the laboratory. Prompt isolation of patients with symptoms of intestinal infections and proper disinfection of their dejecta must be carried out until such time as a definite diagnosis is established. Contacts should be examined bacteriologically and the strictest personal hygiene should be practiced within the household. Convalescents should be examined systematically until the bacteria no longer can be isolated from their excreta. Cheever[151] in particular has called upon the practicing physician to take a more active part in the control of the infections and the education of the general public.

In the United States there is an unfortunate tendency no longer to require stool cultures in the examination of food handlers. When one considers the increasing incidence of reported salmonellosis, one must question the wisdom of such an omission. Without exception, all of the writers who have dealt with the problem of the prevention of salmonellosis have emphasized the necessity of stool examinations, and most of them have insisted that these be frequent. When one considers that salmonellosis has been transmitted through the medium of such foods as coconut,[15] dried yeast,[153] soya milk,[154] and smoked fish[155] in which salmonellae are not indigenous, the role of the human carrier in the processing plant becomes increasingly apparent. The systematic and frequent examination of food handlers should extend not only to workers in food dispensing establishments, but to those in food processing plants as well. Any individual who exhibits any symptoms of intestinal infection should be prevented from handling food until it is established that pathogenic bacteria cannot be demonstrated in his stools. Considering the possibility of transmission of the infections through food, one cannot refrain from noting that it seems rather futile to require a blood test of food handlers for venereal disease, but to make no effort to determine the presence of pathogenic microorganisms in their stools.

The control of hygienic conditions in food processing plants, as well as food-dispensing establishments, must be improved. Galton *et al.*[41] demonstrated that thorough and consistent cleansing of abattoirs and equipment resulted in a marked reduction in the recovery of salmonellae from carcasses and from the environment. It is probable that enforcement of the newly adopted poultry ordinance will result in an improvement of conditions in poultry processing plants. Savage[124] has suggested that the adoption of bacteriological standards for the control of abattoir and food processing establishments would be very helpful. Hardy and Galton[119] cite the successful adoption of coliform organisms as an index of pollution in dairy products and shellfish and, while calling attention to the need for proper concern for the bacteriological environment of meat processing plants, these authors feel that such an index would be overly sensitive at present. MacCready, Reardon and Saphra[2] feel that an approach similar to that employed in dairy products should be applied to other products of animal origin, including fish and poultry. It must be remembered that food habits are changing rapidly in many countries, particularly in the United States. Food technology has made giant strides

within recent years and Savage[179] in particular has called attention to the removal of food preparation from the family kitchen to the large establishment In many instances it seems that methods of control and measures for their enforcement have not kept pace with advances in food processing

More rigid control of products becomes even more important and practical when it is remembered that salmonellae may survive temperatures to which the food products that contain these organisms sometimes are subjected in cooking[11, 154] Since it is possible to pasteurize certain food products effectively[157, 158] or to destroy salmonellae in them through a combination of acidification and mild heating[159] it would not seem unreasonable to adopt and to enforce bacteriological standards for such products Furthermore it is not uncommon for untoward incidents to occur in connection with the newer method of food preservation and preparation these incidents may lead to the multiplication of organisms present in the food and in some instances to the contamination of previously cooked products Large numbers of salmonellae have been found in turkey carcasses that had been frozen thawed and refrozen and which due to improper method of preparation gave rise to a severe outbreak of salmonellosis (Mackel and Payne personal communication) Chicken carcasses that had been thoroughly cooked were contaminated during boning by persons who had prepared the raw carcasses[160] These examples are cited only to call attention to the need of improved procedures and practices in the mass preservation and preparation of foods

All of the writers who have given serious thought to the control of salmonellosis have arrived at similar conclusions Grant[156] Hinshaw and McNeil[169] and Savage[179] all have published excellent reviews dealing with the epidemiology and control of salmonellosis All are agreed that effective control can result only through the cooperation of governmental agencies dealing with health agriculture and food Further it is agreed that salmonellosis should be a reportable disease of both man and animals and that effective reporting and control measures should be enforced The responsibilities of the physician and the health officer as well as of the practicing veterinarian the veterinary officer and the sanitarian are indicated It is only through close cooperation of these individuals and the aforementioned governmental agencies and through education of the public in the fundamental sanitary aspects of the preparation and preservation of foods that control of the infections may be expected

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THE EXOTIC ZOONOSES

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According to Webster s International Dictionary (second edition 1953) zoonosis is defined either as a disease communicable from animals to each other or to man or as a disease due to animal parasites In a broad sense this inclusive definition may be applied to almost any communicable disease of animals regardless of whether it is transmissible to man

Despite this definition the term zoonosis appears only once in the titles of other papers presented in this monograph In some circles there seems to be a feeling of dissatisfaction with the various applications of this term This attitude has been voiced in conversations published papers [1] and discussions In this paper the word zoonosis when not otherwise qualified is used to refer to disease of animals

It is not intended to develop a discussion on the use of the term itself but it is believed desirable to call attention to a recent article by Wagener in which he succinctly reviews the history introduction various applications and modifications of and substitutions for the word zoonosis Wagener s own summary translated is as follows The present significance of zoonosis is investigated The new words anthropozoonosis and zooanthroponosis are discussed with regard to the epidemiological value and the linguistic significance In order to distinguish the pathogenic effect on men (man) and animals diseases of animals infectious for men (man) are zooanthroponosis (zooanthroponoses) anthropozoonosis (anthropozoonoses) are those human diseases which are infectious for animals '

In such a comprehensive monograph as this it is to be expected that there will be some degree of overlapping and duplication of the subject matter Insofar as practicable references to diseases covered by other contributors have been omitted in this paper

The importance of animals to human health has been expressed aptly by Middleton[3] as follows When man domesticated certain lower animals for his personal comfort and gain he assumed the obvious hazard of sharing their diseases So far as is known however animals are reservoirs of many but far from all of the diseases that affect man On the other hand man may be the primary source of some diseases transmissible to the lower animals

As pointed out by Shope [4] there is good indirect evidence to show that swine acquired influenza from man in 1918 An outbreak of influenza virus infection in a ferret colony has been attributed to human sources It appears therefore that man and his influenza viruses constitute a health hazard for at least these two species of lower animals Another example is tuberculosis of the human type which on occasion has complicated efforts to eradicate the disease in animals by means of the tuberculin test [5] Except for occasional

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transitory sensitization to tuberculin infection with the human type of tuberculosis is of little importance in cattle although it may be so in some other species

As has long been known and as again reported recently[6] infection with the human type of *Mycobacterium tuberculosis* may on rare occasions persist for a long time in cattle whether acquired through exposure to human cases or by experimental inoculation Attempts to immunize cattle against bovine tuberculosis[7, 8] by injecting them with the human type of tubercle bacilli usually result in establishment of nonprogressive lesions in one or more organs that are difficult to detect by the most careful autopsy In a few instances it was found that the organism was shed in the milk for various lengths of time without showing detectable lesions in the udder Under such conditions bovine infection with human tuberculosis would be dangerous to consumers of raw milk and could produce slight lesions in suckling calves or render them tuberculin sensitive

In contrast with the e examples is rabies primarily a disease of lower mammals among which an increasingly greater number is being discovered as infected in nature Between these two extremes lies a broad field of interest essentially common both to veterinarians and others concerned primarily with animal diseases and to physicians and public health officials fundamentally responsible for human health Overemphasis of either of these basic interests can only be detrimental to both Consequently increasingly close cooperation among physicians veterinarians epidemiologists and epizootiologists is indicated

Concerning possibilities of disease in man attributable to infection from animals it may be stated that lack of proof of human infection with an agent known to be infectious for animals does not preclude the possibility of such a development to a greater or less extent at some future time Vesicular stomatitis a disease that has occurred sporadically in North America and in portions of Central and South America for decades affecting cattle horses and swine has been found capable of producing an influenza like syndrome in man A few years ago this virus would have been classed generally as noninfectious for man

The specific character of infectious processes in man may be undetermined or even ignored as in the case of animals Also to be considered is the interesting hypothesis that any animal may be attacked by any infectious agent under appropriate conditions

The growing speed and extent of world wide commerce and travel accentuate the danger of the introduction of new disea es whether they be localized in certain regions or be widespread From the broad international viewpoint infection in any quarter of the globe potentially may be carried to any other portion Diseases that are exotic or foreign to one area today may become epizootic or epidemic there in a very short time A notable example is the 1918 pandemic of human influenza

Meyer and Hjarre (cited by Wagener) report at lea t eighty diseases of animals that are transmissible to man Of these only a very small number are of importance from the standpoint of public health

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According to Webster's International Dictionary (second edition 1953) 'zoonosis' is defined either as a disease communicable from animals to each other or to man or as a disease due to animal parasites In a broad sense this inclusive definition may be applied to almost any communicable disease of animals regardless of whether it is transmissible to man

Despite this definition the term zoonosis appears only once in the titles of other papers presented in this monograph In some circles there seems to be a feeling of dissatisfaction with the various applications of this term This attitude has been voiced in conversations published papers,[1] and discussions In this paper the word zoonosis when not otherwise qualified is used to refer to disease of animals

It is not intended to develop a discussion on the use of the term itself but it is believed desirable to call attention to a recent article by Wagener[2] in which he succinctly reviews the history introduction various applications and modifications of and substitutions for the word zoonosis Wagener's own summary translated is as follows The present significance of zoonosis is investigated The new words anthropozoonosis and zooanthroponosis are discussed with regard to the epidemiological value and the linguistic significance In order to distinguish the pathogenic effect on men (man) and animals diseases of animals infectious for men (man) are zooanthroponosis (zooanthroponoses) anthropozoonosis (anthropozoonoses) are those human diseases which are infectious for animals

In such a comprehensive monograph as this it is to be expected that there will be some degree of overlapping and duplication of the subject matter Insofar as practicable references to diseases covered by other contributors have been omitted in this paper

The importance of animals to human health has been expressed aptly by Middleton[3] as follows When man domesticated certain lower animals for his personal comfort and gain he assumed the obvious hazard of sharing their diseases So far as is known however animals are reservoirs of many but far from all of the diseases that affect man On the other hand man may be the primary source of some diseases transmissible to the lower animals

As pointed out by Shope[4] there is good indirect evidence to show that swine acquired influenza from man in 1918 An outbreak of influenza virus infection in a ferret colony has been attributed to human sources It appears therefore that man and his influenza viruses constitute a health hazard for at least these two species of lower animals Another example is tuberculosis of the human type which on occasion has complicated efforts to eradicate the disease in animals by means of the tuberculin test[5] Except for occasional

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part of the nineteenth Century Its prevalence here led to the establishment in 1884 in the United States Department of Agriculture of the Bureau of Animal Industry which was given responsibility for control of this and other animal diseases This disease was subsequently eradicated completely from the United States through slaughter of affected and exposed animals The disease has been eliminated elsewhere from North and South America and Western Europe but it persists in other parts of the world especially in Africa and Australia Although the causative organism variously known as *Asterococcus mycoides Borrimyces pleuropneumoniae* and *Mycoplasma mycoides* is not known to affect man many so called pleuropneumonia like organisms (PPLO) have been discovered in cattle sheep goats dogs rodents and birds as well as in man in various parts of the world[11 19] The classification and nomenclature of this large group of organisms are not firmly established The latest classification based on recommendations of both F A Freundt and D G Edward is used by Merchant and Packer[19]

Glanders primarily a disease of the equidae was once rampant in the United States This disease may now be classed as exotic to that country and to Canada although it persists in many other parts of the world *Malleomyces mallei* causes a serious granulomatous tuberculosis like or ulcerative disease in man that is contracted directly from affected animals Sheep goats dogs and cats occasionally contract the disease naturally A disease etiologically and pathologically resembling glanders namely melioidosis recently has been identified in sheep goats and pigs in the Netherlands West Indies[6] Infection due to the causative organism *M pseudomallei* is said to produce infections in man chiefly in Southeast Asia The presence of the disease which causes polyarthritis emaciation and death should be kept in mind in relation to suggestive unidentified infections of either man or animals

Bluetongue a viral disease that affects sheep primarily and cattle occasionally was believed originally to be confined to the African continent Lately it has spread to other countries in the Middle East and has become a major problem in the United States Insofar as is known man is not susceptible to this infection The disease is an example of what a few years ago would have been classed as exotic

Another example of a formerly exotic animal disease that has become established in the United States presumably through importations of sheep is scrapie As compared with other viral diseases there is an extremely extended incubation period in scrapie—18 months or longer in natural cases This and its insidious nature result in major problems in relation to control and eradication The disease is not known to affect man

In Australia and the United States at about the same time in the late 1930s an acute febrile human illness was given the name Q fever The causative organism *Coxiella burneti* (*R burneti*) has been recovered from *Dermacentor andersoni Amblyomma americanum Haemaphysalis humerosa* and *H bispinosa* and also from cattle sheep goats and various species of wild animals The organism which is shed in milk has been found unusually resistant to common methods of pasteurization Once considered exotic to the United States Q fever must now be classed as an indigenous disease of both man and animal[21]

In a 265 page booklet entitled *Foreign Animal Diseases* [4] compiled by a committee of the United States Livestock Sanitary Association Trenton N J twenty six more or less important exotic animal diseases are listed as follows African swine fever Teschen disease bovine infectious petechial fever contagious bovine pleuropneumonia East Coast fever ephemeral fever infectious infertility of cattle, lumpy skin disease malignant catarrh rinderpest blue tongue contagious agalactia enzootic abortion louping ill Nairobi sheep disease sheep pox, scrapie heartwater nagana Rift Valley fever streptothricosis anthrax foot and mouth disease Venezuelan equine encephalomyelitis fowl plague and Newcastle disease Of these only seven (louping ill Rift Valley fever, Venezuelan equine encephalomyelitis foot and mouth disease sheep pox anthrax and Newcastle disease) are listed as communicable to man

Anthrax and Newcastle disease are included in the list of foreign diseases referred to above despite the fact that both are enzootic to the United States Anthrax is probably included because of the incrimination of imported bone meal as the source of outbreaks in animals and also because imported hides hair and wool are known to be responsible for anthrax in man In 1952 anthrax occurred extensively in animals in some areas of the United States particularly in the Middle Western states It is believed that at least one outbreak was caused by the feeding of imported bone meal containing anthrax spores Since then the United States Department of Agriculture regulations have been amended to provide that only steamed or degelatinized bone meal is eligible for unrestricted entry Such bone meal is commonly referred to as feeding bone meal Other kinds of bone meal are required to go to designated approved industrial establishments for further processing before they are released Bone meals that have been permitted unrestricted entry have been shown on analysis to contain not more than 2 per cent nitrogen and not less than 25 per cent phosphoric acid Such analyses indicate that the bone meal has been processed by cooking at 250 F for at least 1 hour under 20 lb pressure

Newcastle disease of poultry so called because of its appearance in the vicinity of Newcastle upon Tyne in England was not recognized as such in the United States until about 1944 The disease had existed previously in California poultry for a number of years having been identified by the pathologically descriptive name pneumoencephalitis This malady recognized eventually as Newcastle disease spread throughout the United States in a generally mild form describing it from the standpoint of mortality A wide range of species of birds is susceptible [9] and infection has been observed sporadically in man generally as the result of laboratory exposure [10] Although immunologically indistinguishable most strains of this virus in the United States may be differentiated from Asian and European strains on the basis of clinical manifestations pathology and mortality in inoculated birds Outbreaks due to foreign Newcastle disease viruses have been eradicated by destruction of affected and exposed birds

Among the animal diseases there are numerous examples of infections that have been spread through normal traffic channels Bovine pleuropneumonia long a scourge in Europe was introduced into the United States in the early

part of the nineteenth Century Its prevalence here led to the establishment in 1884 in the United States Department of Agriculture of the Bureau of Animal Industry which was given responsibility for control of this and other animal diseases This disease was subsequently eradicated completely from the United States through slaughter of affected and exposed animals The disease has been eliminated elsewhere from North and South America and Western Europe but it persists in other parts of the world especially in Africa and Australia Although the causative organism variously known as *Astero coccus mycoides Borimyces pleuropneumoniae* and *Mycoplasma mycoides* is not known to affect man many so called pleuropneumonia like organisms (PPLO) have been discovered in cattle sheep goats dogs rodents and birds as well as in man in various parts of the world[11-18] The classification and nomenclature of this large group of organisms are not firmly established The latest classification based on recommendations of both F A Freundt and D C Edward is used by Merchant and Packer[19]

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Sheep pox (variola ovina) is an acute highly lethal disease accompanied by formation of elementary bodies It is a serious malady producing high mortality in Asia Southwest Europe and the Middle East While it is a true pox in character it also appears to be peculiar to sheep and has been reported to be distinct from goat pox Evidence that man may be affected is scanty

The arthropod borne encephalitides have acquired major interest among epidemiologists and epizootiologists particularly since identification of equine encephalomyelitis virus in California by Meyer and his associates Since this important finding two immunologically distinct types of the virus eastern and western have been identified in the United States and elsewhere particularly in the Western Hemisphere A third immunologically different type the Venezuelan was identified in 1938[23] Fortunately this disease in the strict sense an exotic zoonosis has not yet been diagnosed in the United States Man is susceptible to all three of these viruses

An endemic encephalomyelitis of horses and possibly of other animals commonly called Borna disease from a locality in Saxony where a severe epizootic occurred from 1894 to 1896 persists in Germany It has a considerably longer incubation period than the encephalomyelitides known in the United States and is apparently distinct from them etiologically It has not been reported to affect man

Rift Valley fever sometimes called enzootic hepatitis an acute mosquito borne viral disease of sheep and cattle has not been identified outside of Africa where it exists particularly in and near the Great Rift Valley It causes abortions in animals and is highly fatal especially to lambs Shepherds farmers butchers and veterinarians often contract the disease in the course of handling infected animals or carcasses The disease in man in whom it has an incubation period of 4 to 6 days resembles influenza The temperature curve resembles that of dengue or yellow fever It is rarely fatal but serious sequelae may occur[24] The virus has been isolated from 8 or more native species of mosquitoes of 2 genera and experimental transmission has been accomplished with some species[25]

Laboratory workers have repeatedly become infected with the virus resulting in illness that cannot be considered inconsequential While the disease has never occurred in animals in the United States or elsewhere outside of Africa there have been a number of laboratory infections in the United States and other countries to which the virus was imported for study Sabin and Blumberg[6] have reviewed cases that occurred in laboratories in various parts of the world including at least 8 cases in the United States In one instance neutralizing antibodies were demonstrable 12 years after the attack

A previously undescribed virus[7] was isolated in 1954 and 1955 from cases of a disease resembling Rift Valley fever that produced abortions in ewes and high mortality in lambs in Wesselsbron Orange Free State Union of South Africa Serum neutralization and complement fixation tests proved this virus to be antigenically different from representative strains of Rift Valley fever virus In fact the disease was identified in ewes previously vaccinated against Rift Valley fever Neutralization tests of cattle sera collected rou

tinely for testing for brucellosis in many parts of the Orange Free State demonstrated antibodies against the W strain of this virus On the basis of unpublished reports it seems that the virus is mosquito borne It is important to note that during the course of study of the virus at the laboratory at Onderstepoort Transvaal Union of South Africa five of the six people engaged in the work became sick but not critically so However muscular pain was experienced for a period of weeks

Louping ill[24] a viral encephalomyelitis particularly of sheep and occasionally of cattle has been known for more than a century in Scotland and northern England more recently the disease has been diagnosed in the Union of Soviet Socialist Republics and Czechoslovakia At times the disease produces heavy losses Serologically the virus has been found to be related closely to that of Russian spring summer encephalitis Similarly louping ill is tick borne chiefly by *Ixodes ricinus* While the sheep disease has never been diagnosed in the United States several human infections have occurred in laboratories in the United States[28] As in sheep the disease in man is diphasic beginning with viremia and concluding after various secondary nervous manifestations Natural infections among humans have also been reported[29, 30]

Foot and mouth disease (FMD) is an acute highly communicable disease affecting almost exclusively cloven footed animals both domesticated and wild It is characterized by the formation of vesicles followed by erosions in the mucosa of the mouth (including the tongue lips cheeks gums dental pad and palate) and sometimes of the gastrointestinal tract It affects particularly the rumen the skin (especially between and above the hoofs) the dew claws the teats and the udder

Very few cases of FMD in man have been authenticated[31, 32] and it has never become a public health problem except as the disease in animals may affect food supplies (milk and meat) it is mentioned here however because of its wide distribution and persistence in animals in Africa Asia Europe and South America From time to time it has occurred in practically every country It has invaded the United States nine times since 1870 the last occasion being in 1929 It was last eradicated from Canada in 1953 and from Mexico in 1954 The only major livestock producing areas presently free of the disease are North America Central America Australia and New Zealand

England has succeeded repeatedly in eradicating FMD only to be reinfected through meat imported from infected countries or in other ways from nearby Europe[33] In countries where the disease becomes epizootic there is an estimated loss of 25 per cent in productivity of cattle swine sheep and goats not considering deaths which are usually 5 per cent or less but may be much greater

Although there has been a total of nine outbreaks of FMD in the United States some very widespread there is not one recorded proved instance of the disease in man in this country Vetterlein[32] has recently reviewed critically the cases reported from 1926 to 1954 He accepts a total of 21 cases as having been appropriately proved Recently Ratner *et al*[34] have described a very unusual lingering case in the Soviet Union from which type A virus was identified The virus is reported to have been obtained on the 25th and 159th days

from lesions on the foot on the 172nd day from the saliva and finally on the 178th day from saliva from so called dry lesions on the palms of the hand and from the feces The character of the lesions and the long duration of the illness are distinctly different from any previously described case

In addition to the diseases and agents previously referred to mention should be made of others

Discovery of yellow fever antibodies in man in regions in South America where *Aedes aegypti* were not universally present led to identification of the so called jungle yellow fever as distinguished from the classic urban type transmitted by that vector Yellow fever antibodies have been found in various species of monkeys other primates and marsupials in these same jungle areas and in the absence of *A aegypti* this type of the disease may thus be classed as a zoonosis that is exotic to North America

Because they are exotic to the United States some other animal diseases not known to affect man but recognized as serious livestock problems should be mentioned Perhaps the most important of these is rinderpest or cattle plague one of the most destructive of all animal plagues which persists in Africa Asia and Eastern Europe Except for a single occurrence in Brazil the disease has never appeared in the Western Hemisphere Although it is essentially a bovine disease it sometimes affects other ruminants and swine In many outbreaks mortality exceeds 90 per cent There is considerable variation in susceptibility among various breeds and types of cattle Accordingly for immunization purposes varyingly virulent virus is used after being adapted to chicken embryos rabbits or goats Some cattle may be so highly susceptible as to require simultaneous administration of antiserum with even the most attenuated virus in order to eliminate losses due to attempted immunization There is no evidence of human infections with this virus

So called Teschen disease [4] a viral encephalomyelitis of swine is reported to be quite widespread in Czechoslovakia where it was first discovered about 1929 near the town of Teschen whence it has spread to other countries in Central and Western Europe Some of the similarities between this disease and poliomyelitis have been explored and there is apparently no relationship between the two viruses [35]

African swine fever [4] is an acute highly fatal septicemic viral disease of swine that symptomatically and pathologically closely resembles hog cholera or as it is designated in many countries other than the United States swine fever The African disease is not known to exist beyond that continent Present information indicates that like hog cholera the disease is not transmissible to man

Wild bush pigs or wart hogs have been found to be symptomless carriers of the virus of African swine fever Domestic swine frequently contract the disease through contact with such feral animals Biologicals that protect against hog cholera have been found ineffective as preventives of African swine fever Indeed no effective means of prophylaxis other than avoiding contact with wild pigs or wart hogs and the eradication of affected domestic herds has yet been developed

The broad scope of the title of this paper would permit continuation beyond

the space allotted however the diseases omitted here or skipped over in a manner that may appear cursory are covered elsewhere in these pages As has been pointed out by Hagan[44] in addition to the human health hazard animal diseases also affect the welfare of man through their serious economic effects It has been estimated[17] that in the United States death losses among livestock from all causes in 1954 amounted to 1 5 million cattle 2 5 million calves 4 million sheep 10 5 million swine 235 million chickens and 7 2 million turkeys These figures do not reflect the whole toll since they do not include losses in other species feed labor and capital investment nor in untold numbers of newborn animals It is estimated that 20 to 35 per cent of the pigs born never reach weaning age Internal parasites have been estimated to exact 400 million dollars annually from incomes from livestock Some of these total losses involve diseases dangerous to man but many do not

Before concluding it is considered appropriate to mention an important aspect of the animal disease problem On October 8 1952 supplemented May 12 1953 notices were sent to laboratories research institutions and individuals studying animal diseases to advise that under regulations of the Department of Agriculture it is necessary to obtain authorization by the Secretary of Agriculture for the importation and interstate transportation of certain animal disease organisms and vectors[81] This notice which was widely published in many scientific journals and other publications directed attention to the inherent danger of such movements and to the urgent need for precautions *against the spread of animal disease*

While the department has the responsibility for safeguarding our livestock and poultry by the prevention and control of domestic diseases as well as those of foreign origin it is not the intention to interfere unduly with the normal movement of specimens to diagnostic laboratories and research institutions or the transfer of organisms and viruses indigenous to this country between research workers and laboratories For example permits are not required for the interstate transportation of members of the *Pasteurella* group *Salmonella* group *Clostridium* group Newcastle disease virus of mild virulence the viruses of such diseases as fowl pox hog cholera and swine influenza or of canine distemper Permits are required in advance for the interstate movement of such agents as Venezuelan equine encephalomyelitis virus highly virulent strains of Newcastle disease virus *Plasmodium berghei* the viruses of bluetongue or scrapie vesicular disease viruses and any other foreign animal disease viruses organisms or vectors that may have gained entrance into the United States accidentally or otherwise Permits are required for the importation of any *organisms or vectors that may transmit diseases to our* animal and poultry population

It is evident that absolute protection against the introduction and dissemination of foreign diseases is virtually impossible however every reasonable precaution should be taken toward this end In order to protect the nation s livestock industry the Secretary of Agriculture is empowered to promulgate rules and regulations which may be modified in accordance with changing conditions and requirements[39]

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tions caused by Listeria in ruminants swine and men are meningoencephalitis septicemia and abortion

Since Nyfeldt isolated Listeria from humans with a disease indistinguishable from infectious mononucleosis[4] numerous other cases have been reported[43 44 49] Listeria infections usually do not give a positive Paul Bunnel reaction and produce a relatively high infant mortality while infectious mononucleosis is usually benign and the affected individual recovers without specific treatment

Conjuctivitis with or without mononucleosis is another manifestation of Listeria infection This has occurred as a result of laboratory infection[9] and a granulomatous type has been reported in a group of poultry processors[4] The ability of Listeria to produce conjunctivitis in rabbits and guinea pigs makes it a useful diagnostic tool[9]

Septicemia is probably the most commonly occurring form of listeriosis in man[61] The resulting involvement of the lymph glands and the reticuloendothelial system stimulates the production of monocytes thus causing confusion in differentiating the disease from infectious mononucleosis Monocytosis is not always present and in these cases a fever leukocytosis and swollen liver and spleen are predominant symptoms

A disease of the newborn described as a granulomatous septicemia has been described by Potel[4 63] It is highly fatal to the infant but curiously enough it does not seriously affect the mother Often the mothers deny any symptoms compatible with listeriosis Thorough questioning frequently establishes some influenzalike symptoms a few days before occurrence of the abortion or premature birth Slight chills light lumbar pains and a slight elevation of temperature usually are all that can be ascribed to the infection in these mothers If the infants survive the first septicemic stage they may develop meningitis[64] While the majority of Listeria meningitis cases occur in the young it is by no means confined to children[45] Kaplan has listed numerous cases of meningitis due to Listeria and many are in the adult age group[68]

Therapeutic agents have been evaluated in both animals and man[1 23 67 69] and the following assumptions can be made from the results obtained and reported in recent literature The antibiotics of the tetracycline group are the drugs of choice for the treatment of listeriosis but penicillin and the sulfonamides in combination proved to be almost as effective An excellent summary of human Listeria cases treated with various therapeutic agents has been published by Seeliger[45] The *in vitro* sensitivity test gives some indication of the agent of choice Certainly chemotherapy and antibiotic therapy have increased the odds in favor of recovery from malignant listeriosis when such treatment is begun early and with sufficient amounts to bring blood levels up to an effective listeriostatic or listeriocidal concentration Untreated cases of meningitis polyserositis and overt septicemic disease usually are fatal

Vaccination of animals against listeriosis has been attempted[79 70 81] The results of these trials have not met with any great degree of success The economic value of sheep and cattle populations together with public health considerations indicate strongly that these investigations should be continued

LISTERIOSIS A POTENTIAL PUBLIC HEALTH PROBLEM

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Listeriosis is a little known infrequently recognized but widespread bacterial disease of man and other warm blooded creatures The causative agent, *Listeria monocytogenes* has been recovered from man other mammals and birds in the following countries Australia [1] Belgium Brazil [3] Canada [4] Ceylon [5] Denmark [6] Germany [7] Uraguay [8] Austria [9] Argentina [10] England [11] Finland [1] France [13] The Netherlands [14] India [15] Israel [16] Japan [17] New Zealand [1] Norway [18] Poland [7] Sweden [19] Switzerland [0] the Union of Soviet Socialist Republics [21] South Africa [3] Czechoslovakia [4] Turkey [5] and the United States [6] This indicates that, geographically, this is certainly a ubiquitous organism Many species of animals have served as host for the bacterium *Listeria monocytogenes* has been isolated most often from sheep [1] [6-8] cattle [7] [9] and man [30-38] but occasionally from foxes [39] chinchillas [40] lemmings [41] rabbits [11] swine [14] horses [18] canaries [4] hares [4] chickens [43] [44] goats [9] ducks [45] geese [5] eagles [46] turkeys [47] heath cocks [48] dogs [49] [50] deer [51] cats [51] field mice [5] ferrets [53] house mice voles [16] gerbils [3] skunks [54] and raccoons [55] Laboratory animals such as the white mouse [3] and guinea pig [8] also have been shown to be susceptible to *Listeria*

Murray Webb and Swann isolated the organism in 1924 from laboratory rabbits with a fatal septicemic disease Because of the monocytosis produced in this species these investigators tentatively called the organism *Bacterium monocytogenes* [11] Three years later Pirie found a similar disease in South African rodents that was caused by the same type of organism which he called *Listerella hepatolytica* [3] English investigators agreed to call the organism *Listerella monocytogenes* but because this name is a homonym of a mycetozoan Pirie suggested *Listeria monocytogenes* which is now the accepted name [57]

Morphologically the organism is a Gram positive nonacid fast nonspore forming rod There is a tendency for the organisms to arrange themselves into V s or into a palisade formation when the smear is made from solid media The bacteria stain evenly and are 0 5 to 0 6 by 1 0 to 2 0 μ in size Short but sometimes long threadlike chains may be observed in the rough phase of growth Capsules are not demonstrable Motility can be demonstrated in young (6-hour) glucose broth cultures but is more pronounced at 25 C after 24 hours incubation The peritrichously flagellated organisms have a characteristic tumbling motion

An interesting and important cultural characteristic of *Listeria* is its frequent failure to grow when cultured from fresh tissue This unusual behavior may account to some extent for failures to diagnose listeriosis in human disease It has been found that refrigeration of the original necropsy specimens and periodic subculturing will increase the number of isolations obtained [58]

Listeriosis in sheep and cattle usually is recognized readily and the etiological agent is frequently recovered without difficulty The main clinical manifesta

spread might be venereal in nature cannot be overlooked. *Listeria* has been recovered from the urethra of some young adult males[74] and we must not forget that the meconium of infected prematurely born infants is swarming with *Listeria*, and that isolations have been made from the placenta, the cervix and the vagina of human and animal females.[23, 31, 45, 51, 78]

Two recent reports of human infections following direct contact with animals moribund with listeriosis strongly suggest that this mode of transmission is possible.[76, 77] *It may be, however, that the source of infection for the individuals concerned was common to both the livestock and the animal husbandryman.*

Nonpasteurized milk may contain *Listeria*.[19] Seeliger points out that in East Germany, where milk was still being rationed in 1955, unpasteurized milk was frequently illegally purchased directly from farmers. Human cases of listeriosis are frequent in this area, as are animal infections. The sale of unpasteurized milk on the black market may be a source of infection for humans.[55]

Another interesting observation was a number of cases among shoe repairmen.[48] These individuals might have become infected from the dirt and manure brought in on shoes to be repaired. One can speculate that in this instance the disease was contracted via the airborne route by means of the dust created during the repair process. It is entirely possible that unclean hands could have contaminated food eaten by the individuals, or that tacks customarily carried in the mouths of repairmen might be a means of spreading contamination.

There are four serotypes of *Listeria*, and among these are several biotypes.[73, 79] All serotypes and biotypes can and do attack man, poultry, and mammals. Serotype 4 has been divided into 4a and 4b.[45] The most prevalent serotypes are 1 and 4b.[45] There does not appear to be any association of serotypes either to the species of animal or to the clinical type of disease. The primary value *of serotyping and biotyping is for epidemiological purpose. For example*, serologic surveys show more rural children with agglutinating antibodies than are present in urban children.[31]

Agglutinating antibodies against *Listeria* differ in amount. For example, Lang demonstrated that about 65 per cent of several hundred children 3 to 10 years of age had antibodies against somatic or flagellar antigens, with titers ranging from 1:40 to 1:160. Only one third of older children had such titers.[31] The specificity of agglutinating titers is open to question, since some sera spontaneously agglutinate antigen, and *Listeria* shares antigens with other bacteria such as the staphylococci and *Streptococcus fecalis*.[19] This being the case, agglutinating titers must be interpreted with caution and, preferably, compared with another type of serologic test using a different antibody system, such as the complement fixation test.

The identification of any organism found in the spinal fluid or blood of patients is desirable; likewise, cultures from necropsy specimens should be identified. *Listeria* cultures are sometimes discarded because the bacteriologist, being unfamiliar with the organism, occasionally considers it to be a diphtheroid

Whether or not an infectious disease constitutes a public health problem depends upon its appearance within a population the severity of the disease, and the nature of its epidemiology Therefore by definition listeriosis constitutes such a problem because we do not know its frequency of occurrence nor do we understand the epidemiology of the disease We do know that it occurs frequently in livestock that it is often fatal to both animals and man and that its role in human infections requires clarification

The geographical distribution and host range of the disease have been mentioned Other factors such as season sex and age distribution persistence and serotypes are of interest in solving the problem

There is a definite seasonal distribution in animal infections [26, 7, 9, 71] The greatest number of cases occurs during the winter and spring months It would appear that this is not true of human infections *Listeria* cultures referred to the Communicable Disease Center laboratory for identification or verification were slightly more numerous during the cool months A breakdown by months gives the following figures January 8 February 2 March 6 April 4 May 4 June 3 July 4 August 4 September 6 October 10 November 7 and December 7 Case reports published[30, 33, 35, 37, 65, 66, 79] that give the dates of admission to the hospital show the following distribution January 0 February 3 March 0 April 0 May 1 June 0 July 0 August 3 September 2 November 1 and December 1

It is possible that neither the sex distribution nor the age distribution of the disease in animals and man is significantly different It must be emphasized however that in human infection mothers frequently have no definite symptoms pointing to listeriosis and consequently may be overlooked as a source of infection for the infant It may be that sex does not influence the course of the disease but that age is a factor in determining whether the course of the disease is fatal that is the younger the animal or human the less favorable the prognosis The sex distribution of listeriosis in livestock is influenced by husbandry practices For example the number of male animals employed usually is 1 bull for 25 to 50 cows and 1 ram to 25 to 35 ewes Therefore the small number of cases in noncastrated male animals is easily explained

Direct contact seems to play a part in the spread of infection among animals Those animals that shared a common feed rack but that were separated by a fence from infected animals did not become infected while those in the same pen did become diseased Attempts to recover the organism from straw litter drinking water and feed were unsuccessful However it was found that *Listeria* could survive for long periods of time under barn conditions The shortest period of survival was six weeks on straw and wood shavings and the longest time was twenty six weeks on rabbit food pellets [77] Other studies have shown that it is possible to infect animals through seeding their drinking water with cultures of *Listeria* [77]

Human infections while usually sporadic have occurred as epidemics The exact mechanism of spread other than by direct contact was not apparent *Listeria monocytogenes* was recovered from the blood and also from the throat washings of 8 of 26 individuals with overt illness [73] The possibility that human

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We may conclude therefore that listeriosis is a public health problem in the United States and elsewhere It is necessary that the physician and medical bacteriologist be constantly aware that *Listeria* is widespread that the premature infant and the aborted fetus may be due to a congenitally acquired agent that does not always manifest itself in an overt illness of the mother that all ages and both sexes are susceptible that animal contact is not necessarily a factor in the epidemiology of listeriosis that the older the individual the greater is the chance of survival from this particular disease except in the ages 60 years or more and that the tetracyclines are efficacious in the treatment if started early

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THE PUBLIC HEALTH IMPORTANCE OF ANIMAL TUBERCULOSIS

By R. J. Anderson
Communicable Disease Center, Public Health Service, Atlanta, Ga.

Although tuberculosis is common to a wide range of animals, mammals, birds, and even fish and reptiles, only cattle are important hosts where human health is at risk. Tuberculosis in swine is a problem to the farmer, to be sure, for his hogs may be condemned at slaughter as unfit for human consumption. Pet animals sometimes have tuberculosis, but the likelihood of their transmitting it to human beings is rather remote. Since cow's milk is a staple of the diet in the United States, however, the bovine type of tuberculosis which humans can contract by ingesting milk from tuberculous cows has long been a concern of public health in this country.

The control of tuberculosis in cattle has had a remarkable history in the United States. Controversy surrounded the advocacy of tuberculin testing and slaughter of reactors forty years ago. There was both skepticism about the communicability of bovine tuberculosis to animals or to man and a wide belief that the destruction of tuberculin reacting cattle would be ruinous to the agricultural economy. In spite of these obstacles the proponents of tuberculin testing and slaughter of reactors were able to establish a national campaign in 1917, supported by a congressional appropriation. The campaign rapidly grew to full force and, in the ensuing forty years, hundreds of millions of cattle have been tested and the reactors destroyed.

The success of this work in reducing the percentage of tuberculin reactors among cattle tested is a matter of record. From a high of almost 5 per cent in 1918, reacting cattle reached a low in 1952 of 0.11 per cent. Slightly higher rates in recent years (0.15 per cent in 1956) are attributed to more aggressive attempts to find sources of tuberculosis by tracing reactors to their herds of origin and testing those herds. Even 1.5 reactors per 1000 cattle tested constitutes a very low rate compared with those found in many other areas of the world. In some countries the control of bovine tuberculosis is only beginning.

As the practice of pasteurizing milk has become more widespread it has given added protection to the population against exposure to bovine tuberculosis. Today about 95 per cent of the milk consumed by the urban population of the United States is pasteurized. Nine states and 64 cities require pasteurization of the milk that is sold in their jurisdiction.

The effect of these programs in lowering the prevalence of bovine tuberculosis in humans is difficult to measure. Statements that the bovine type of tuberculosis now plays an insignificant role in this country are common in current medical literature, but no data are available to substantiate them because presence of the causative strain of bacillus is not required in tuberculosis reporting in the United States. In the 1920s and 1930s a number of authors studied the relative frequency of bovine infection and reported widely ranging percentages in the various groups of patients. In discussing B. Mollers' summary of the literature up to 1927, from which he found that 11.1 per cent of

2277 cases studied by various authors were due to bovine infection Rich[1] points out that such a composite must be considered in light of the fact that the incidence of bovine infection varies widely from area to area that it has been greatly reduced in some places since 1927 and that studies usually have been made where there was some reason to believe that the incidence of bovine infection was high a set of conditions that would tend to inflate the figures on bovine infection Since the United States has always had a lower rate of tuberculosis in cattle than most of the rest of the world application of Mollers 11 1 per cent or of Price s 10 per cent (based on a 1939 study of 18 000 cases reported in the literature from all over the world) would be inappropriate here

Since bovine infection affects children more often than adults and usually lodges in parts of the body other than the lungs deaths from extrapulmonary tuberculosis among children are often used as a rough index of the incidence of bovine infection Price points out that the reduction in mortality from extrapulmonary forms of tuberculosis parallels the eradication of tuberculosis in cattle Such a parallel however is less convincing when one considers that the reduction in mortality from extrapulmonary forms has also paralleled the reduction in mortality from pulmonary tuberculosis In the absence of exact data the question of whether we have less tuberculosis of the spine bones and joints and less scrofula in the United States because of the control of tuberculosis in cattle or because of factors that have brought about lowered pulmonary tuberculosis death rates remains a matter of speculation Better social and economic conditions and control measures such as more aggressive case finding the isolation of patients in sanatoria and better medical care all would certainly be expected to bring about a decline in the incidence of the nonpulmonary as well as the pulmonary forms of the disease

I do not wish to give the impression that I am minimizing the importance of the excellent work that has been done in the United States to eradicate tuberculosis in cattle I do believe however that we are now at a point where assessment of its public health importance is in order Consideration of the situation raises these challenging questions for both medical and veterinary epidemiologists To what extent are bovine type tubercle bacilli infecting humans and causing disease in them? What is the source and the mechanism of transmission? Is the program of eradication of tuberculosis in cattle to be justified in terms of its effect on human tuberculosis or is its importance related chiefly to the agricultural economy? Indeed is a program that is sufficient for the agricultural economy adequate for the control of human tuberculosis? What indices are valid for decisions about the relaxation cessation or intensification of present programs?

When a disease is fairly widespread effective control programs can be based upon empirical judgments of where disease exists and how much of it there is With success in lowering prevalence comes the necessity for focusing efforts directly on the remaining problem in order to use control facilities economically

Our first question is perhaps the most important To what extent are bovine type tubercle bacilli infecting humans and causing disease in them? The culturing and strain typing of bacilli are now routine practice in many

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not announce itself in sudden dramatic onset but often develops slowly and can go unrecognized for long periods Its public health importance must be evaluated by some means more definite than opinion The relatively much greater prevalence of tuberculosis of human origin overshadows and obscures the absolute indices of the extent of bovine tuberculosis infection This denies to us the use of the mass of data on tuberculosis in humans that is readily available in assessing the present-day importance and nature of bovine type infection We are therefore forced into specific inquiry if such is our objective

In the history of any disease-control program particularly those directed against widespread infections the later phases during which eradication is almost in sight often present unusual difficulties In the beginning when a method of control is first available enthusiasm is high and this enthusiasm will continue as long as the method produces demonstrable results As the problem diminishes control measures may either be neglected or be carried on routinely without critical examination of their effectiveness Too often re-evaluation and remeasurement of the problem and of the methods in use against it remain long overdue I suggest that the time is ripe for such a re evaluation of the public health importance of animal tuberculosis

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tuberculosis hospitals and clinics, especially in connection with studies of the atypical acid fast bacteria (the so called yellow bacilli and Battey type organisms) that are being recovered in many cases diagnosed as tuberculosis However since both tuberculosis hospitals and public health clinics are concerned chiefly with pulmonary disease even when routine typing is done it probably can give little information about the extent of bovine infection Canvassing of general hospitals children s hospitals and physicians such as orthopedists or dermatologists who may encounter tuberculosis of the bones or skin would be a necessary additional productive line of inquiry In every case where bovine bacilli are isolated thorough investigation of the source of infection might uncover otherwise unsuspected remaining reservoirs of the disease or throw light on the mechanism of transmission and on the significance of cattle infection for humans

From a practical standpoint, this type of investigation would be most appropriate in communities where tuberculin testing of cattle is sporadic Objective review of the figures on cattle testing by states suggests that there is considerable variation in coverage both geographically and in point of time Furthermore in many rural areas where dairy farming is not the major emphasis of agriculture families customarily keep one or several cows to supply their own needs How often are these family cows tested? Is their milk customarily pasteurized before the family drinks it? It seems to me that the possibility that bovine tuberculosis is still harbored in such areas would bear investigation not only through examination of hospital records and inquiry about physicians experience but also by use of the tuberculin test on the children in such areas By and large human tuberculin sensitivity has been found to be low in the rural districts of the United States as we should expect from the fact that fewer cases of disease are found there However some children in almost every rural area do react to tuberculin and it would seem both practical and profitable to investigate carefully their opportunities for exposure to bovine infection

In any discussion of bovine tuberculosis the suggestion always arises that this form of the disease occurs more often than we think and that it goes unrecognized The occasional instance in which bovine tuberculous infection of the gastrointestinal or genitourinary organs is diagnosed raises in the epidemiologist s mind the question are other cases occurring that are never diagnosed accurately? Are we so unsuspicious of the existence of bovine infection that we fail to notice it? Does it produce subclinical illness that rarely receives medical attention? This too is a matter not for speculation or for argument but for investigation The tuberculin test and history of possible exposure to bovine tuberculosis could perhaps provide leads in the differential diagnosis of obscure abdominal complaints

The public health importance of animal tuberculosis it seems to me cannot be measured with the information we now have We think that bovine tuberculosis in humans is rare The cases that have come to the attention of the Communicable Disease Center since 1950 cases confirmed by laboratory study can be counted on the fingers of one hand However this communicable disease which exists in animal hosts closely associated with man does

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morbidity[9] In the rural population of the United States it is estimated that approximately 70 to 80 per cent of fungus infections involving the exposed parts of the body are caused by zoophilic dermatophytes with animals serving as vectors of these diseases In the urban population it is estimated that 10 to 30 per cent of such infections are produced by zoophilic dermatophytes with animals playing the role of vectors of infection

It is evident that animal ringworm presents definite public health implications Recognizing the importance of this problem and the many voids in our over all knowledge of this entity the Mycology Laboratory Communicable Disease Center Chamblee Ga in cooperation with the Veterinary Public Health Section of the Center initiated a long range animal ringworm study in 1953 An integral part of this study is a survey[10] to determine among other things the fungi involved and the prevalence of these infections in our lower *animal population This survey has been conducted with the cooperation of* veterinarians from all parts of the United States who submit clinical materials from animals with skin lesions for mycologic study In addition a companion survey was carried out on wild animals trapped in southwest Georgia by personnel of the Newton Field Station of the Communicable Disease Center It is noteworthy that the wild animals did not show any manifest cutaneous abnormalities at the time the specimens were collected

The purpose of this report is to present and to discuss our findings in these studies for the 18 month period from January 1956 to June 1957

Materials and Methods

The clinical materials received for study consisted of hairs and/or skin scrapings Upon arrival at the Mycology Laboratory each specimen was examined under a Wood s light for evidence of fluorescence This procedure was followed by a direct microscopic examination to detect the presence of dermatophytes The microscopy was carried out in 10 per cent potassium hydroxide mounts Each specimen was then cultured on a special selective isolation medium[11] containing the antibiotics cycloheximide * penicillin and streptomycin and incubated at room temperature (25 C) For the isolation of *Trichophyton verrucosum* the medium was enriched with thiamine and cultures held at 37 C[1] The cycloheximide was omitted from the medium for the isolation of *Trichosporon cutaneum* since that organism was found to be inhibited by this antibiotic Each specimen was inoculated into a minimum of three tubes of media and all cultures were held for one month before reporting any as negative

Results

Domestic Animal Ringworm Study

As noted on TABLE 1 during the period covered by this report specimens from 2361 domestic animals with skin lesions were examined These materials were submitted by veterinarians from 35 states in all sections of the country

* Actidione manufactured by The Upjohn Co Kalamazoo Mich Trade names are used for identification purposes only Mention of them does not constitute an endorsement by the Public Health Service

RECENT DEVELOPMENTS IN ANIMAL RINGWORM AND THEIR PUBLIC HEALTH IMPLICATIONS

By William Kaplan Lucille K. Georg and Libero Ajello
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Introduction

The ability to break down keratin is an important characteristic of the dermatophytes. *In vivo* these fungi are parasites of the keratinized tissues of the body, such as the epidermis, the hair, and the nails, and they produce alterations of the structures invaded. These alterations, together with attendant immunological reactions, are clinically classified as ringworm.

Both man and the lower animals are susceptible to infection by ringworm fungi. However, the individual dermatophyte species show varying degrees of host specificity. Some are parasites of man and these rarely, if ever, infect lower animals; others are primarily animal pathogens that are found to produce human disease on occasion. Thus, on the basis of host preference or host adaptation, the ringworm fungi have been divided into two groups. In one category have been placed the human pathogens or anthropophilic organisms, and in the other, the animal pathogens or zoophilic dermatophytes. While it is true that some of the human and animal pathogens have been recovered from the contaminated environment of infected hosts[1-3] such apparent saprophytism appears to be secondary to their parasitism of man or animals. Until recently, nearly all of the known dermatophytes have been placed in one or the other of these two groups.

Recent findings, however, point to the necessity of establishing still another category of dermatophytes, one that would draw its members from among the keratinophilic soil inhabiting fungi that have the ability, under certain conditions, to invade the keratinized tissues of human and animal hosts and there to produce disease. Members of this group are essentially saprophytes and probably serve along with other keratinophilic organisms in the microbiological breakdown in soil of keratin into simple elements. To date, only two species, *Microsporum gypseum*[4, 5] and *Keratinomyces ajelloi*[6-8] have been demonstrated to belong in this third category. Thus, from an epidemiological standpoint, it is logical at this time to consider the existence of these three groups of ringworm fungi: the anthropophilic or "human type" organisms, the zoophilic or "animal type" organisms, and the "geophilic" or soil organisms.

Members of the three groups of dermatophytes are involved in human disease and each type presents distinct public health problems. As might be anticipated, the vast majority of ringworm infections in man are caused by anthropophilic organisms. Such conditions as "athlete's foot" and ringworm of the groin and nails are common conditions affecting large segments of our population. They are caused by anthropophilic dermatophytes and animals have not been shown to play a role in the epidemiology of these diseases. However, if one excludes these common infections and considers only ringworm of the exposed parts of the body, such as the scalp, face, neck, and extremities, then the zoophilic organisms appear as important contributors to human

morbidity [9] In the rural population of the United States it is estimated that approximately 70 to 80 per cent of fungus infections involving the exposed parts of the body are caused by zoophilic dermatophytes with animals serving as vectors of these diseases In the urban population it is estimated that 10 to 30 per cent of such infections are produced by zoophilic dermatophytes with animals playing the role of vectors of infection

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TABLE 1
DOMESTIC ANIMAL RINGWORM SURVEY JANUARY 1956 TO JUNE 1957
DERMATOPHYTES ISOLATED BY HOST SPECIES

Host animal species	Number specimens examined	Organisms isolated: *Microsporum canis*	*M. gypseum*	*M. audouinii*	*M. distortum*	*Trichophyton mentagrophytes*	*T. equinum*	*T. verrucosum*	*T. rubrum*	*Keratinomyces ajelloi*	*Trichosporon cutaneum*†	Total positive	Per cent positive
Dog	1659	252	68	1	1	41			1	3		368	22.1
Cat	524	193	4			3						200	38.1
Cattle	58							29				29	50.0
Horse	56	2	12				4					18	32.1
Monkey	21	10			4						1	15	71.4
Chinchilla	12	1				2						3	25.0
Guinea pig	9					6						6	66.0
Rabbit	8											0	0.0
Donkey	2						1	1				2	100.0
Sheep	5											0	0.0
Malabar squirrel	1									1		1	100.0
Chicken	1											0	0.0
Parakeet	4											0	0.0
Chimpanzee	1											0	0.0
Total	2361	358	84	1	5	52	5	30	1	1 + 3*	1	642	27.2

* *K. ajelloi* strains not shown to be pathogenic
† *T. cutaneum*

Pathogenic fungi were recovered from animals residing in 29 different states. Of the total number of specimens studied, 642 (27.2 per cent) were found to be positive by culture.

As shown on TABLE 1, 9 dermatophyte species and a *Trichosporon* were isolated and identified from the clinical materials submitted. These were *Microsporum canis*, *M. gypseum*, *M. audouinii*, *M. distortum*, *Trichophyton mentagrophytes*, *T. equinum*, *T. verrucosum*, *T. rubrum*, *Keratinomyces ajelloi* and *Trichosporon cutaneum*. As might be expected, the majority of these dermatophytes are specialized zoophilic parasites of lower animals. However, 2 of the species, *M. gypseum* and *K. ajelloi*, are soil organisms and 2 others, *M. audouinii* and *T. rubrum*, are essentially human pathogens. As a background to the presentation of our findings relative to the individual host animal species it would be well to discuss briefly each of the organisms encountered.

Microsporum canis (*M. felineum*, *M. equinum*, *M. lanosum*). This fungus has been shown by a number of investigators both in the United States and elsewhere to be the common cause of ringworm in cats and dogs. Our findings further confirm this fact and, in addition, reveal that *M. canis* is also an important cause of ringworm in monkeys. Although *M. canis* is primarily a zoophilic dermatophyte, human infections by this fungus are not uncommon. The organism is readily transmitted to man by animals, particularly by cats and dogs, and is responsible for the majority of the animal-type ringworm infections in the urban human population of the United States.[13] In rural areas this agent presents less of a public health problem. Person-to-person

transmission of this fungus can occur however the organism appears to lose its infectivity after a few such human passages Thus large scale epidemics in man are not caused by *M canis* Rather isolated cases or group infections form the pattern observed the total number of human cases being a reflection of the morbidity in animals The Wood s light is a useful tool in the detection of this infection in both man and animals However this diagnostic aid does have its limitations as evidenced by our findings that only 30 per cent of the *M canis* infected hairs fluoresced

Microsporum gypseum (*M fulvum Achorion gypseum*) *M gypseum* has been shown to be a common soil fungus Ajello[4] reported the recovery of this agent from 37 (31 9 per cent) of 116 soil samples collected from various sites in Tennessee and Georgia Subsequent analyses of foreign and domestic soils by several workers[5 8 14] have resulted in equally frequent isolations Although *M gypseum* is infectious for man it does not appear to be an important human pathogen In several surveys[15-17] of human ringworm this fungus has been found to be responsible for only 0 7 (2 1 per cent) of the cases studied Until recently animal infections have been considered to be equally uncommon A review of the world literature by Ajello[4] in 1953 revealed that up to that time only 61 authenticated lower animal infections by this organism had been re corded However the results of our studies would indicate that *M gypseum* is a not uncommon cause of ringworm in animals As noted on TABLE 1 *M gypseum* was isolated from 84 animals with clinical ringworm during the period covered by this report This fungus is often referred to as a zoophilic dermato phyte in the belief that animals serve as reservoirs of infection for man It is possible that occasional animal to-human transmission does occur However in those instances where the infection has been diagnosed concurrently in man and his animal contacts the possibility that soil served as a common source of infection for the hosts involved was not considered or investigated The wide spread distribution of this fungus in soil together with its demonstrated ability to exist in nature as a free living agent[18] would indicate that *M gypseum* is essentially a saprophyte that can under certain conditions assume a parasitic existence Consequently this mold would fall into the category of geophilic dermatophytes and should not be regarded as an animal type fungus Al though some workers have reported fluorescence of *M gypseum* infected human hairs under the Wood s light we have never observed this phenomenon in our animal cases Thus the Wood s lamp does not appear to be an appropriate tool for the detection of this infection in animals

Microsporum audouinii This fungus is considered to be a human pathogen with animals playing no role in the epidemiology of the infection *M audouinii* is the agent most commonly implicated in epidemic ringworm of the scalp in children in the United States In recent years outbreaks involving thousands of school children have been reported from all sections of the country Al though this agent is essentially a human pathogen there are recorded in the literature[19-23] five authenticated infections in lower animals In three of these cases dogs in one a monkey and in one a guinea pig were involved These few cases of course do not indicate that animals serve as unsuspected reser voirs of this infection yet they are significant to the extent that lower animals

have been proved to be susceptible to this agent It is possible although perhaps unlikely that future studies may change our concept of the part played by animals in the natural history of this agent As noted on TABLE 1 we recovered *M audouinii* from a dog with clinical ringworm The animal involved was a 10-week old boxer that had been acquired by a family that had two children under dermatologic care for tinea capitis Infected hairs collected from this dog were observed to fluoresce under the Wood s light Subsequent to the period covered by this report we again isolated *M audouinii* from a dog with clinical ringworm [4] This animal was a 6 month old terrier that had been recently purchased by a family that included a young boy under treatment for ringworm of the scalp In this case *M audouinii* was also recovered from the human It would appear that in these two episodes man transmitted the infection to the lower animals

Microsporum distortum This new species of *Microsporum* was described in 1954 by Di Menna and Marples [5] as a cause of human disease in New Zealand The epidemiological features of the outbreaks in that country suggested to these workers that animals were the source of the human infections However these investigators did not recover this fungus from lower animals As shown on TABLE 1 we isolated *M distortum* in four instances from monkeys and once from a dog that had been in contact with one of the infected simians [26] All five animals had clinical ringworm These recoveries demonstrated that *M distortum* does indeed infect lower animals in nature and that it is present in the New World The monkeys involved had been recently imported from Central America While conclusive proof was not obtained to show that *M distortum* had been brought into the United States by the monkeys the evidence is sufficiently suggestive in nature to warrant further investigations into this possible avenue of importation The public health implications of this infection are evidenced by the fact that six humans that had been in contact with three of the simians were reported to have developed ringworm The demonstrated ability of this agent to infect a variety of host species points to the possibility of its establishment in the lower animal population of this country Hairs invaded by *M distortum* fluoresce under the Wood s lamp

Trichophyton mentagrophytes var *granular* (*T gypseum T asteroides T granulosum*) This variety must be differentiated from the downy type of *T mentagrophytes* that is the human pathogen commonly found to cause athlete s foot [27] The granular variety is a zoophilic dermatophyte that is transmitted by animals to man Human infections with this agent are encountered principally in rural areas In one study [8] *T mentagrophytes* var *granular* was shown to be responsible for approximately one half of the cases of suppurative ringworm in humans in rural areas of Michigan This fungus is pathogenic for a diversity of animal species both domestic and wild including rodents and larger mammals [9]

Trichophyton equinum As the species name would imply this fungus is primarily a pathogen of horses Epizootics of equine ringworm caused by this fungus have been described from the United States [9] and other parts of the world [10-3*] In some of the outbreaks infections have been reported among human contacts However such cases have been few in number a fact that

indicates minimal pathogenicity for man In this country *T equinum* has not been generally recognized as a distinct species In the manual of Conant et al[33] it is listed as a synonym of *T mentagrophytes* However nutritional and morphologic studies[34] recently carried out in our laboratory have led us to recognize that *T equinum* is a valid species and is not a variant of *T mentagrophytes*

Tricophyton verrucosum (*T faviforme T album T discoides T ochraeceum*) This dermatophyte is the principal cause of cattle ringworm in the United States and other parts of the world[35 36] Occasional infections occur in other animal species[9] Man is susceptible to infection by this fungus and *T verrucosum* appears to be an important cause of suppurative ringworm in humans residing in rural areas In the study carried out in Michigan[23] approximately one half of the cases of suppurative ringworm in rural residents were shown to be caused by this fungus

Trichophyton rubrum (*T purpureum*) This dermatophyte is an important cause of ringworm of the feet and nails of man in the United States and in other parts of the world In recent years there have been indications that human infections with this agent have been increasing[37] The reasons for this trend are not too well understood and have been the subject of considerable speculation There is no evidence however that animals are involved in the epidemiology of this disease Although *T rubrum* is generally regarded as an obligate anthropophilic dermatophyte natural infections in animals can occur Chakraborty Ghosh and Blank[38] reported the first recovery of this agent from lower animals As noted on TABLE 1 during the period covered by this report we isolated *T rubrum* from a dog with clinical ringworm The animal involved was a one year old boxer the owner of which had a mild case of tinea pedis of several years duration The owner had the habit of rubbing the dog with his bare feet *T rubrum* was recovered from the owner s feet Thus it would appear that in this case man transmitted the infection to a lower animal[39]

Keratinomyces ajelloi This fungus was first described in 1952 by Van breuseghem[6] who recovered the organism from Belgian soil Subsequent studies[4 8] carried out in the United States and elsewhere have shown this agent to be a common soil inhabiting fungus Although man and lower animals are constantly exposed to this agent in nature there had been no reports of infection with this species until 1956 During that year we recovered *K ajelloi* from a case of ringworm in a Malabar squirrel[40] In this instance we were able to demonstrate tissue invasion and were successful in reproducing the disease in guinea pigs with the strain isolated As noted on TABLE 1 this fungus was also recovered from three dogs with skin lesions However tissue invasion was not demonstrated which would indicate that in these instances the organism most probably was present on the skin as a contaminant To date there have been no reports of human infections with this interesting fungus Although cases of ringworm due to *K ajelloi* are apparently very rare it is possible that future studies may show this fungus to be an occasional cause of disease

Trichosporon cutaneum Members of this genus are not dermatophytes They are the cause of a fungus disease of the hair known as white piedra In

fected hairs are characterized by the formation of nodules along their shafts Most of the cases in man have been reported from South America Asia and Europe[33] The paucity of reported human cases from the United States[41] would indicate that white piedra is rare there Piedra has been reported in lower animals This condition has been observed in a horse[42] and in clinical materials from chimpanzees in Germany[43] The chimpanzee cases however appear to be black piedra a disease caused by an ascomycete *Piedraia hortai* The organism responsible for the equine case is not known As noted in TABLE 1 we recovered a *Trichosporon* from a monkey[44] with clinical white piedra This is the first such authenticated infection to be reported from a simian The animal involved had recently been imported from Latin America This interesting finding suggests the possibility that piedra may not be uncommon in the simians of Latin America

It is evident from the above comments that the dermatophytes encountered in our survey show varying degrees of host specificity Some are pathogenic for a diversity of animals others parasitize only a few species It is interesting to note that with the exception of *K ajelloi* all fungi to date have been shown capable of infecting man With these points in mind let us examine some of our findings with respect to the individual host animal species

Infections in dogs As noted on TABLE 1 specimens from 1659 dogs were received for laboratory study Of this number 368 (22 1 per cent) were found to be positive by culture In order of frequency the three principal dermatophytes encountered were *M canis* in 252 cases (69 per cent of the infections) *M gypseum* in 68 cases (18 per cent of the infections) and *T mentagrophytes* in 41 cases (11 per cent of the infections) The dermatophytes *M audouini* *M distortum* and *T rubrum* each were recovered once and the soil organism *K ajelloi* was recovered three times

There was no apparent difference in the clinical picture produced by the various dermatophytes encountered Approximately 80 per cent of the infections occurred in dogs less than two years of age The most common lesions observed by the attending veterinarians in this series were circular scaly areas of alopecia with or without crust formation located on the head and extremities Kerion development was reported in some cases and in others the clinical picture simulated eczema

Of the 368 dogs shown to have ringworm there was a history of skin lesions among members of 36 owner families In 30 of these episodes *M canis* was recovered from the dogs involved The dermatophytes *M audouini* *M distortum* *M gypseum* and *T rubrum* were each isolated in one instance from the other dogs *T mentagrophytes* was recovered in two cases from the pets involved Unfortunately in only five episodes did the attending physicians culture the human cases or submit clinical materials to a public health laboratory for mycologic study Consequently evidence that the humans were suffering from the same infection as their pets must be considered presumptive

Infections in cats Specimens from 524 cats were submitted for laboratory study (TABLE 1) Of this number 200 (38 1 per cent) were found to be positive by culture The principal dermatophyte encountered in this series was *M canis* The fungus was isolated in 193 cases (96 5 per cent of the cases) *M*

gypseum and *T mentagrophytes* were recovered in 4 cases and 3 cases respectively [45, 46] Approximately 65 per cent of the infections occurred in cats less than one year of age approximately 80 per cent of the infections occurred in felines less than two years of age The lesions observed by the attending veterinarians varied from scattered loss of hair to diffuse scaling and alopecia The areas of the body involved were principally the head and the extremities

The public health importance of feline ringworm was emphasized by the fact that among the owners of the 200 cats found to be infected there was a history of skin lesions among members of 63 of the families In all such cases *M canis* was recovered from the felines involved As far as could be determined in 12 instances in which lesions were reported among human contacts the cats involved had been recently purchased from breeding establishments in 10 instances the animals involved were strays that had been recently brought into the home in 2 episodes the animals had been recently obtained from friends and in 14 cases the felines had been permitted to run out of doors at will In the remaining cases it was not possible to obtain additional information It should be pointed out that in only 3 of these episodes were the humans cultured by the attending physicians and *M canis* was recovered by the dermatologists from these patients Therefore evidence that in the remaining instances the humans were infected by the same agent as the cats must be considered presumptive

Infections in cattle Clinical materials for laboratory study were received from 58 head of cattle including dairy and beef breeds (TABLE 1) Of this number 29 (50 0 per cent) were found to be positive on culture The only dermatophyte encountered was *T verrucosum* Although ringworm in cattle is most commonly observed during the fall and winter months when cattle are kept in barns three outbreaks were reported in the summer months The lesions most commonly observed were raised crusted areas circular in outline located principally on the head neck and tail regions

In 3 outbreaks of cattle ringworm lesions were reported among members of the owner families Unfortunately the attending physicians did not culture the human cases On the basis of clinical appearance however the lesions were diagnosed as animal type ringworm In one such episode a farmer his wife and their 4 children ranging in age from 6 months to 10 years developed skin lesions It is of interest to note that the 6 month old infant who had never been in contact with the animals was the first to become infected and that the father who had the greatest contact with the infected stock was the last to develop lesions This episode again demonstrates the possibility of indirect spread of this infection [47]

Infections in horses During the year 1956 clinical materials from 56 horses were examined (TABLE 1) Of this number 18 (32 1 per cent) were found to be positive on culture Three different dermatophyte species were isolated They were *M canis* *M gypseum* and *T equinum*

The first organism *M canis* is an important cause of equine ringworm in some parts of the world [48] In this country however little information is available concerning the prevalence of this infection in horses A review of the literature shows only one previously reported case of *M canis* ringworm in

a horse in the United States[49] Our two isolations of this dermatophyte represent the second and third times that *M canis* has been demonstrated to cause equine ringworm in the United States

The second organism, *M gypseum*, does not appear to be a common cause of equine ringworm A review of the world literature by Ajello[4] in 1953 disclosed that up to that time only 50 such infections had been recorded All of the cases had been described from Europe, with the exception of 2 from Madagascar Prior to 1956 this disease had not been reported in horses in this country Since that time we have recovered *M gypseum* from 12 horses with clinical ringworm Eight of the animals were involved in an epizootic of ringworm[50] and 4 appeared as isolated cases Thus *M gypseum* does not appear to be an uncommon equine pathogen in this country

The third fungus *T equinum* was recovered from horses in four instances In our series the clinical picture produced by the three dermatophytes was basically the same The appearance of the lesions varied with the stage of the disease Early lesions appeared as raised crusted areas that tended to be circular in outline As the disease progressed the crusts fell off leaving scaly areas of alopecia Where widespread loss or breakage of hair had occurred the affected areas presented a moth eaten appearance

Although there was close contact between the infected horses and humans over a long period of time there was no evidence of transmission of disease from equines to man

Infections in monkeys Specimens from 21 monkeys were received for study (TABLE 1) All the simians were of species indigenous to Central and South America Ten of the animals were found to be infected with *M canis* four with *M distortum* and one with *Trichosporon cutaneum*

The lesions most commonly seen were scaliness of the skin with varying degrees of alopecia In two cases a more severe tissue reaction was observed as evidenced by the development of kerions The parts of the body most commonly involved were the extremities and tail

Five of the simians shown to be infected with *M canis* and three with *M distortum* were reported to have transmitted the disease to human contacts However the attending physicians did not culture the human cases to determine the etiological agent The diagnoses of ringworm were made on the basis of clinical appearance More intensive studies are indicated to determine the full public health importance of ringworm in monkeys

Infections in chinchillas Specimens from twelve chinchillas were received for laboratory study (TABLE 1) The dermatophytes *M canis* and *T mentagrophytes* were isolated in one and two cases respectively

Blank[51] presented evidence that would indicate that *T mentagrophytes* is a cause of fur slipping a skin disorder of economic importance to chinchilla breeders In one case where we recovered *T mentagrophytes* the animal involved was a member of a group of three hundred chinchillas the majority of which were reported to have skin lesions The infected animals had scaly patches of alopecia located most commonly on the nose behind the ears and around the base of the tail In this outbreak the owner and her son were reported to have developed skin lesions as a result of handling the animals

Infections in guinea pigs Nine guinea pigs maintained at the Communicable Disease Center were cultured and *T mentagrophytes* was isolated in six instances (TABLE 1) This agent had previously been incriminated in an epizootic of ringworm involving several thousand guinea pigs at the Communicable Disease Center [62] In our few cases the animals showed scaly erythematous patches of alopecia on the tip of the nose around the eyes and at the base of the tail None of the individuals handling these animals developed skin lesions

Infections in other animal hosts *Keratinomyces ajelloi* was isolated from a Malabar squirrel Clinical material was received from 2 donkeys (TABLE 1) One of the animals was found to be infected with *T equinum* and the other with *T verrucosum* In addition specimens from 8 rabbits 5 sheep 1 chicken 4 parakeets and 1 chimpanzee were cultured In none of these cases were ringworm fungi recovered

Wild Animal Ringworm Study

During the period covered by this report hair specimens from 2350 wild animals were cultured (TABLE 2) Twenty one different animal species including rodents and larger mammals are included in this series Of the total number of specimens examined *M gypseum* was recovered in 3 instances once

TABLE 2
WILD ANIMAL RINGWORM SURVEY JANUARY 1956 TO JUNE 1957
FUNGI ISOLATED BY HOST SPECIES

Host animal group	No. specimens examined	Fungi isolated		
		Microsporum gypseum	*Trichophyton mentagrophytes*	Rodent *Microsporum**
Opossum (*Didelphis marsupialis*)	379		6	11
Raccoon (*Procyon lotor*)	352			1
Skunk (*Mephitis mephitis*)	246			2
Gray fox (*Urocyon cinereoargenteus*)	87			
Cottontail rabbit (*Sylvilagus floridanus*)	205			1
Cotton rat (*Sigmodon hispidus*)	336		1	57
House mouse (*Mus musculus*)	91			1
Gray squirrel (*Sciurus carolinensis*)	3			
Red fox (*Vulpes fulva*)	18			
Cotton mouse (*Peromyscus polionotus*)	18			
Fox squirrel (*Sciurus niger*)	51			
Marsh rabbit (*Sylvilagus palustris*)	4			
Domestic cat (feral) (*Felis domestica*)	26			
Bobcat (*Lynx rufus*)	61			
Old field mouse (*Peromyscus polionotus*)	61	2	1	
Harvest mouse (*Reithrodontomys sp*)	4			
Roof rat (*Rattus rattus*)	76			
Golden mouse (*Peromyscus nuttalli*)	1			
Wood rat (*Neotoma floridana*)	1			
Otter (*Lutra canadensis*)	1			
Norway rat (*Rattus norvegicus*)	129	1	1	1
Total	2350	3	9	74

* A new species of *Microsporum* Pathogenicity never demonstrated

from a Norway rat (*Rattus norvegicus*), and twice from old field mice (*Peromyscus polionotus*) The dermatophyte *T mentagrophytes* was isolated from 6 opossums (*Didelphis marsupialis*), 1 cotton rat (*Sigmodon hispidus*) 1 old field mouse (*P polionotus*), and 1 Norway rat (*R norvegicus*) In addition as shown on TABLE 2 we obtained 74 isolates of a fungus that at this time is simply referred to as the 'red *Microsporum* This organism was recovered from 11 opossums (*D marsupialis*) 1 raccoon (*Procyon lotor*) 2 skunks (*Mephitis mephitis*), 1 cottontail rabbit (*Sylvilagus floridanus*) 57 cotton rats (*S hispidus*) 1 house mouse (*Mus musculus*) and 1 Norway rat (*R norvegicus*) The red *Microsporum* is considered to be a new dermatophyte species [53] and a manuscript describing this fungus is now being prepared for publication A similar organism has been recovered from soil in Idaho and Washington by Cooke [54] and in Michigan by Aiello [55] It should be pointed out again that none of the animal hosts from which this organism was recovered showed any skin lesions In addition the Wood s light and direct microscopic examination of the clinical materials failed to reveal any evidence of infection Furthermore all experimental attempts to demonstrate pathogenicity of the red *Microsporum* for man and animals have been unsuccessful Thus the potential public health implications of this new fungus species remain to be determined

It is of interest to note that the pathogen *Trychophyton mentagrophytes* was recovered from wild rodents and opossums In a previous report[56] similar findings have been recorded The fact that feral animals, particularly rodents of the type commonly found on farm premises have been repeatedly demonstrated to be carriers of this dermatophyte may have important epidemiological implications Large numbers of rodents are commonly found in barns feed storage bins and other sites on farms and rodent carriers of *T mentagrophytes* may serve as indirect sources of human infections by contaminating the environment with elements of this fungus

Discussion and Conclusions

It is obvious that the term ringworm denotes a clinical entity rather than a specific infection A number of distinct fungus species can cause dermatophytoses in animals as well as man Each organism presents its own epidemiological and epizootiological implications Thus measures designed to control outbreaks of ringworm in human and animal populations must be based upon knowledge of the organisms involved and their ecology Therefore reliable mycologic support would be imperative for any well founded control program

Our studies have shown that ringworm in animals is a common dermatological condition with definite public health implications Nevertheless this entity has been largely ignored by public health workers in the United States to date To a great degree this situation reflects the neglect of the whole field of medical mycology in the past In recent years however there has been a marked increase of interest in the fungus diseases of man and animals and it is encouraging to note the many advances made in our over all knowledge of these infections It is to be expected that as our understanding of animal ringworm infections accumulates and their public health implications are fully

appreciated control programs will be developed in the United States Such programs already have been initiated in Great Britain [57]

Summary

Based on recent findings a modified classification of the dermatophytes from an epidemiological standpoint is presented and described This classification includes three groups the anthropophilic or human type dermatophytes the zoophilic or animal type and the geophilic or soil inhabiting fungi Members of the third group are free living keratinophilic fungi commonly found in soil which have the ability under certain conditions to parasitize human and lower animal hosts and produce disease The public health importance of these three groups of organisms is reviewed

The results of the animal ringworm survey conducted during the period of January 1956 to June 1957 are presented and discussed Clinical materials from 2361 domestic animals with skin lesions were examined Of these animals 642 (27 2 per cent) were found to be positive by culture Nine dermatophyte species and a *Trichosporon* caused these infections They were *Microsporum canis M gypseum M audouinii M distortum Trichophyton mentagrophytes T equinum T verrucosum T rubrum Keratinomyces ajelloi* and *Trichosporon cutaneum*

Clinical materials from 2350 wild animals trapped in southwestern Georgia also were cultured These animals did not show any apparent cutaneous abnormalities The dermatophyte *M gypseum* was recovered in 3 cases *T mentagrophytes* in 9 instances and a new species of *Microsporum* in 74 other cases

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Etiology

In contrast to the modern trend in virology in which numerous agents are found in nature without diseases to call their own cat scratch fever is somewhat old fashioned In this case we have a definite disease but no established cause for it although by processes of elimination a virus of some type is the most likely suspect

Mollaret *et al*[229] reported the isolation of a virus as the cause of the disease but confirmation of this work has not been possible to date Numerous attempts to isolate such an agent in tissue culture by animal injection and by chick embryo investigation have failed with great regularity

The consensus has been that the agent is probably a member of the psittacosis lymphogranuloma group Three factors have lent seeming credibility to this hypothesis First the disease is characterized by subacute granulomatous lesions of the lymph nodes second the sera of patients who have recovered from the disease frequently show the presence of complement fixing (CF) antibodies for lygranum antigen and third one of the most common viruses present in the domestic feline is the agent of feline pneumonitis a member of the psittacosis-lymphogranuloma group Admittedly these criteria form an extremely shaky base for the theoretical selection of an etiological factor but the nature of the agent has been assumed by the majority of investigators

Recently however Armstrong *et al*[230] have suggested that there is no reason to assume that the causal agent of cat scratch fever is a member of the psittacosis-lymphogranuloma group of viruses They report that the reaction to lygranum CF antigen is not a significant factor in diagnosis Many cases have no rise in titer and some individuals in the older age groups may have a titer with no history of clinical disease This contention is also emphasized by the work of others[231 232]

The presence of inclusion or elementary bodies has been discussed in detail by Mollaret *et al*[234] The finding of basophilic intracytoplasmic bodies was considered by these workers as evidence of specific cellular reaction to the agent of benign lymphoreticulosis or direct virus growth Intracellular bodies were found in both the peripheral primary lesion and in affected lymph nodes Basophilic inclusion bodies are commonly found in the macrophages of the lesion[234] but are also present in the same pathological tissues of other diseases including tularemia tuberculosis and bacterial adenitis The absence of inclusion bodies has been noticed in cases of cat scratch fever by Kalter Prier and Prior[235] and it is now generally accepted that there are no specific intracellular bodies associated with the lesions

The possibility that cat scratch fever may be a manifestation of some other specific disease has been considered but all evidence to date leads to the conclusion that it is a complete and separate pathological entity Beaumont and Gill[1] have suggested that the disease was the same as rat bite fever but the organisms associated with the latter have not been isolated from active lesions

CAT SCRATCH FEVER

By J. E. Prier

Merck Sharp & Dohme, West Point, Pa.

The close relationship existing between the human and the domestic animals and the interdependence of these species are matters of record in the sociological and scientific literature that forms the history of civilization. At one time such association was close physically as well as economically. Man shared his living quarters, his water and, in some instances, even his food with the species of agricultural importance, and this cooperative existence frequently resulted in equal sharing of some diseases.

Only a few remnants of this type of existence remain today. It is reported, at least through that medium of modern electronics that has become an integral part of existence in the United States, that the cowboy still lives with his horse, the shepherd with his flock, and Tarzan with the beasts of the jungle. For the most part, however, modern husbandry dictates that the livestock upon which our way of life depends be maintained separate and apart from the human domicile. Most of the diseases that were common to the species have been brought under control. Progress has been made in these areas.

However, in the relationships of man to animals other than agricultural species, the situation is somewhat different. The home, which was well on its way to becoming a human sanctuary, has been invaded by a curious array of nonhuman species. Also, the communities of the world are overrun with the domestic feline, a species of dubious value but definite detriment.

The most common disease transmitted to man by the domestic cat, aside from insomnia, is a condition that has been given such names as nonbacterial regional lymphadenitis, benign inoculation lymphoreticulosis, and cat bite fever, and L. Foshay of the University of Cincinnati, Cincinnati, Ohio, is credited with the name "cat scratch fever." As a recognized clinical entity this disease is new, the majority of cases having been reported within the past six years. The number of diagnosed cases is increasing, although there is no direct evidence that the disease actually occurs more frequently now than in previous years. Some cases have been tentatively diagnosed in retrospect, one as early as 1925.

It is generally conceded that the disease was first recognized in the United States, although this was unpublished, by Foshay in 1932. In 1935 the disease was described in Great Britain by Beaumont and Gill[1] and by Brown.[2] Following the descriptions by Debre *et al.* in 1950[3-5] and by Mollaret *et al.*[6-12] in France, and Greer and Keefer (1951)[13] in the United States, numerous case histories and research reports appeared in the literature of the United States and other countries.[14-73] There is little doubt that the disease is one of clinical and public health importance, and that probably the present incidence will persist or increase until more information regarding etiology and prophylaxis becomes available.

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Source of Infection

The most frequent predisposing factor to the clinical case of cat scratch fever is a skin injury produced by the claw of a cat. The age of the offending animal is not important. Cat bites and, occasionally, dog bites also have been incriminated. At least one case has been attributed to contact with urine. Other sources of infection have been a porcupine quill, wooden splinters, thorns, insect bites, and abrasions.[235, 236] It is not known, of course, whether such vectors were contaminated with the infecting agent by cats, either directly or indirectly.

It is reasonable to assume that cats may carry a transmissible agent in their upper respiratory system or oral cavity. The feline habit of frequently licking the paws results in almost constant contamination of the claws with saliva. It remains to be demonstrated, however, that clinically normal cats are carriers of a viral agent. With the present knowledge of orphan viruses in many species, it becomes plausible to assume that such a possibility exists.

Cats that have been incriminated definitely in the transmission of cat scratch fever have been examined in detail. It is usual that the animals involved are normal, even to detailed clinical and laboratory examination, but such normal cats are capable of subsequent transmission of the disease; even multiple family outbreaks have been noted.[235, 237] Thompson and Miller[238] in discussing a case of cat scratch fever encephalitis noted that an involved cat died several days after onset of the primary lesion. There were insufficient facts available to assume that this death was related to the capacity of the animal to transmit cat scratch fever.

Detailed post mortem examinations of cats involved in the production of cat scratch fever cases have been done by the author. In no case has there been identified a lesion or disease syndrome that could be connected with the animal's ability to transmit disease. In at least one case it was demonstrated that the animal was capable of inducing cat scratch fever at the time of post mortem inspection. This cat, received from a patient who had recently developed the disease, accidentally scratched an individual at the time of examination. As a result, a mild but typical case of cat scratch fever developed, including initial lesion, lymphadenopathy, and subsequent development of a positive reaction to a skin test antigen.

In general, therefore, the sources of infection shed no light on the problem of etiology. In fact, there is no evidence indicating that the cat acts other than merely as a common mechanical carrier of the disease agent.

Transmission to Experimental Animals

Information to date tends to indicate that man is peculiar in his susceptibility to benign lymphoreticulosis. Mollaret[239] described the transmission of the disease to monkeys. He injected a suspension of lymph node obtained from a human case and produced both a local lesion and regional lymphadenopathy. Unfortunately, however, sufficient confirmation of this work has never been presented.

Peterman[229] in discussing a probable case that occurred in 1925 noted that materials from the lesions of the patient were injected into guinea pigs. The

injected animals developed lung lesions that reportedly bore a histological similarity to those of the naturally infected human

For the most part the use of laboratory animals in the study of cat scratch fever has been entirely unsatisfactory Mice guinea pigs rats rabbits chicks suckling animals and hamsters have all been used in attempts to elicit a regular response to injection of supposedly infective materials Dogs and cats have also been used without success Studies employing schemes of blind passage in guinea pigs were futile

It is perhaps of particular interest that cats of any age are totally refractory to the injection of purulent material obtained from the lesions of acute cases Such animals do not develop and do not naturally carry antibodies against the lymphogranuloma group of agents Also they do not react to the skin test antigen which is a result of diagnostic significance in the human patient

The Clinical Disease

The primary lesion The majority of cases develop a lesion at the site of inoculation Although the time of appearance is variable in the extreme it usually appears 3 to 14 days after the injury One case[19] had an apparent incubation period of 62 days It is a relatively benign lesion being red slightly raised and papular in appearance It may proceed to a minor ulceration with a small necrotic core Small multiple papular eruptions have been observed in some cases and infrequently a transient localized measles like eruption occurs

Systemic symptoms The majority of patients exhibit a febrile period some temperatures exceeding 104 F The course of the fever is quite variable With some an undulating curve is noted others may have a low fever of short duration (1 or 2 days) still others may have a relatively high fever that persists for 4 days or longer About one third of the patients are afebrile

Other general symptoms include anorexia malaise weakness chills and aches Many cases are hospitalized although this acute phase subsides within a few days in most instances

Lymphadenopathy Aside from the primary skin lesion the most common change associated with the disease is a regional lymphadenopathy Varying degrees of swelling are noted and nodes of nearly any area may be involved Most typically the immediate draining lymph gland is affected first and subsequently the secondary nodes become involved Thus in a scratch or bite about the hand swelling of the epitrochlear node is seen after several days and then the axillary nodes are involved Submandibular suprascapular preauricular and cervical nodes are enlarged following injuries about the face head and neck Such nodes may also be secondarily involved following inoculations of the upper limbs Hilar and mesenteric nodes have been involved in a few cases but the pathogenesis of such conditions is not clear

The affected nodes are swollen and produce varying degrees of pain and tenderness As the disease progresses the enlargement may cease and the node becomes indurated In other instances the nodes enlarge suppurate and frequently require surgical drainage The pus derived from such lesions is thick gray and bacteriologically sterile Nodes recede in size in 2 to 6

weeks, though some may require a longer period to return completely to normal Surgical removal of the affected node does not seem indicated commonly

Involvement of the nervous system By far the most serious sequela of cat scratch fever is involvement of the central nervous system (CNS) Such complications were first brought to light by Stevens[241] In describing the disease in a 13 year old boy he recorded convulsions stupor coma and other evidence of CNS disturbance In the same year Debre[36] reported similar symptoms in a case in France These cases were actually diagnosed in retrospect Thompson and Miller[238] recognized the first case during the acute stages of the disease The central nervous symptoms did not become apparent until one month after the initial injury Sufficient cases of this type have not been recognized to ascertain without doubt that CNS involvement is an important and not altogether rare result of infection with the agent of benign lymphoreticulosis

Other sequelae In the examination of patients presumably ill of cat scratch fever other involvements have been observed In some cases however it is not quite clear that the symptoms and lesions are referable to the primary disease Rather they may be coincidental disease syndromes

Belber Davis and Epstein[4] reported the association of thrombocytopenic purpura with a typical regional lymphadenopathy type of cat scratch disease Five weeks after the initial injury and following a course of regional lymphadenopathy, generalized petechiae in the skin hematuria bleeding gums and blood in the stools characterized a well defined disease process The condition was sufficiently severe to require splenectomy After recovery a cat scratch skin test was done and *supposedly due to an allergic response a brief episode* of purpura occurred

At least two cases of bone involvement have been reported due to or at least associated with cat scratch fever[43] One patient developed a granulomatous osteomyelitis on a digit following a severe cat bite The second individual developed a lesion on the right ileum a presumptive diagnosis of eosinophilic granuloma which healed spontaneously in nine months The latter case had a typical syndrome of lymphadenopathy preceding development of the bone lesion

Lesions of the eye have been reported though these are not of frequent occurrence Daniels and MacMurray[44] have described a conjunctivitis and preauricular adenitis due possibly to entrance of the infection via the nasopharynx Parinaud's oculoglandular syndrome has been discussed by Cassady and Culbertson[15, 246] and others[23, 69] as a probable result of direct conjunctival infection This syndrome nonspecific in etiology may also result from infection with the tubercle bacillus yeast *Pasteurella tularensis* viruses and *Leptothrix* Pronounced enlargement of the regional lymph nodes is associated with a severe acute conjunctivitis

Pathology

The specific pathology is associated either with the primary lesion or the involved lymph node *Grossly the primary lesion is a papular eruption*

microscopically it is a superficial circumscribed ulceration with an underlying acute inflammatory process[2,15]

Lymph nodes appear either as small indurated specimens or enlarged firm masses encapsulated and with or without evidence of suppuration On section white necrotic nodules are apparent beneath the capsule bearing a distinct resemblance to tubercles Cut surfaces usually have a pearl gray to tan background

The histopathological appearance of lymph nodes has been classified into three groups by Kalter Prier and Prior[22] (1) acute caseous (2) acute necrotizing and (3) epithelioid cell In all types a capsular thickening with marked chronic inflammatory cell reaction is noted Large numbers of eosinophils frequently are present among the plasma cells and lymphocytes in the capsular region and giant cells are sometimes present in various parts of the lesions

The acute caseous lesion is essentially a soft tuberculoid granuloma There is a large central amorphous caseous mass around which is found a peripheral zone of epithelioid cells Foreign body type giant cells also may be present in this area Lymphocytes are likewise present and many of the epithelioid cells show degenerative changes These lesions are not readily distinguishable from reactions induced by the tubercle bacillus

Acute necrotizing lesions are characterized by a central core of polymorphonuclear leukocytes There is no well-defined layer of epithelioid cells at the periphery and no definite demarcation from surrounding tissue A marked tendency for fusion of adjacent lesions is observed

Epithelioid cell lesions are essentially collections of epithelioid cell granuloma toma No evidence of caseation of cellular degeneration is present The cells are large with vesicular nuclei acidophilic nucleoli and a relatively large portion of cytoplasm Marked germinal center hyperplasia is also observed in this type of involvement

Diagnosis

Tentative diagnosis is possible by consideration of the history of a cat bite or scratch followed by the development of the typical picture of lymphadenopathy and gradual spontaneous regression of symptoms and lesions More difficulty is encountered of course when atypical syndromes occur

I do not suggest however that full attention need not be given to complete examination for diseases that may produce similar clinical pictures such as tularemia brucellosis lymphogranuloma venereum tuberculosis and neoplastic disease of the lymphatic system Experience indicates that overenthusiasm in the diagnosis of cat scratch fever may be as misleading as lack of consideration of the malady Consequently an adequate laboratory diagnostic procedure may include in addition to complete hematological studies serological examination for *Brucella* tularemia infectious mononucleosis and lymphogranuloma and skin tests for histoplasmosis blastomycosis and tuberculosis

Supporting evidence for diagnosis can be obtained by histological examination of lymph nodes if surgical intervention is indicated

If positive serologic examination for antibodies to lygranum antigen may support the diagnosis (TABLE 1) It must be emphasized however that

weeks though some may require a longer period to return completely to normal. Surgical removal of the affected node does not seem indicated commonly.

Involvement of the nervous system. By far the most serious sequela of cat scratch fever is involvement of the central nervous system (CNS). Such complications were first brought to light by Stevens.[241] In describing the disease in a 13 year old boy he recorded convulsions, stupor, coma, and other evidence of CNS disturbance. In the same year, Debre[3b] reported similar symptoms in a case in France. These cases were actually diagnosed in retrospect. Thompson and Miller[238] recognized the first case during the acute stages of the disease. The central nervous symptoms did not become apparent until one month after the initial injury. Sufficient cases of this type have not been recognized to ascertain without doubt that CNS involvement is an important and not altogether rare result of infection with the agent of benign lymphoreticulosis.

Other sequelae. In the examination of patients presumably ill of cat scratch fever, other involvements have been observed. In some cases, however, it is not quite clear that the symptoms and lesions are referable to the primary disease. Rather, they may be coincidental disease syndromes.

Belber, Davis, and Epstein[4] reported the association of thrombocytopenic purpura with a typical regional lymphadenopathy type of cat scratch disease. Five weeks after the initial injury and following a course of regional lymphadenopathy, generalized petechiae in the skin, hematuria, bleeding gums, and blood in the stools characterized a well defined disease process. The condition was sufficiently severe to require splenectomy. After recovery, a cat scratch skin test was done and, supposedly due to an allergic response, a brief episode of purpura occurred.

At least two cases of bone involvement have been reported due to, or at least associated with, cat scratch fever.[43] One patient developed a granulomatous osteomyelitis on a digit following a severe cat bite. The second individual developed a lesion on the right ileum, a presumptive diagnosis of eosinophilic granuloma, which healed spontaneously in nine months. The latter case had a typical syndrome of lymphadenopathy preceding development of the bone lesion.

Lesions of the eye have been reported, though these are not of frequent occurrence. Daniels and MacMurray[44] have described a conjunctivitis and preauricular adenitis due possibly to entrance of the infection via the nasopharynx. Parinaud's oculoglandular syndrome has been discussed by Cassady and Culbertson[45, 46] and others[3, 59] as a probable result of direct conjunctival infection. This syndrome, nonspecific in etiology, may also result from infection with the tubercle bacillus, yeast, *Pasteurella tularensis*, viruses and *Leptothrix*. Pronounced enlargement of the regional lymph nodes is associated with a severe acute conjunctivitis.

Pathology

The specific pathology is associated either with the primary lesion or the involved lymph node. Grossly, the primary lesion is a papular eruption

microscopically it is a superficial circumscribed ulceration with an underlying acute inflammatory process[25]

Lymph nodes appear either as small indurated specimens or enlarged firm masses encapsulated and with or without evidence of suppuration On section white necrotic nodules are apparent beneath the capsule bearing a distinct resemblance to tubercles Cut surfaces usually have a pearl gray to tan background

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weeks though some may require a longer period to return completely to normal Surgical removal of the affected node does not seem indicated commonly

Involvement of the nervous system By far the most serious sequela of cat scratch fever is involvement of the central nervous system (CNS) Such complications were first brought to light by Stevens [41] In describing the disease in a 13 year old boy he recorded convulsions stupor coma and other evidence of CNS disturbance In the same year Debre[36] reported similar symptoms in a case in France These cases were actually diagnosed in retrospect Thompson and Miller [38] recognized the first case during the acute stages of the disease The central nervous symptoms did not become apparent until one month after the initial injury Sufficient cases of this type have not been recognized to ascertain without doubt that CNS involvement is an important and not altogether rare result of infection with the agent of benign lymphoreticulosis

Other sequelae In the examination of patients presumably ill of cat scratch fever other involvements have been observed In some cases however it is not quite clear that the symptoms and lesions are referable to the primary disease Rather they may be coincidental disease syndromes

Belber Davis and Epstein [42] reported the association of thrombocytopenic purpura with a typical regional lymphadenopathy type of cat scratch disease Five weeks after the initial injury and following a course of regional lymphadenopathy generalized petechiae in the skin hematuria bleeding gums and blood in the stools characterized a well defined disease process The condition was sufficiently severe to require splenectomy After recovery a cat scratch skin test was done and supposedly due to an allergic response a brief episode of purpura occurred

At least two cases of bone involvement have been reported due to or at least associated with cat scratch fever [43] One patient developed a granulomatous osteomyelitis on a digit following a severe cat bite The second individual developed a lesion on the right ileum a presumptive diagnosis of eosinophilic granuloma which healed spontaneously in nine months The latter case had a typical syndrome of lymphadenopathy preceding development of the bone lesion

Lesions of the eye have been reported though these are not of frequent occurrence Daniels and MacMurray[244] have described a conjunctivitis and preauricular adenitis due possibly to entrance of the infection via the nasopharynx Parinaud s oculoglandular syndrome has been discussed by Cassady and Culbertson[245] [46] and others[3] [59] as a probable result of direct conjunctival infection This syndrome nonspecific in etiology may also result from infection with the tubercle bacillus yeast *Pasteurella tularensis* viruses and *Leptothrix* Pronounced enlargement of the regional lymph nodes is associated with a severe acute conjunctivitis

Pathology

The specific pathology is associated either with the primary lesion or the involved lymph node Grossly the primary lesion is a papular eruption

TABLE 2
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Total number tested	Skin test reaction	No	Per cent	Scratched by cat		Intimate contact with cat		No known contact with cat		Adenopathy	
				No	Per cent	No	Per cent	No	Per cent	No	Per cent
Patients 250	Positive	210	84 0	135	65 0	70	33 0	5	2 0	210	100
	Negative†	25	10 0	5	20 0	15	60 0	5	2 0	25	100
	Doubtful	15	6 0	15	100 0	—	—	—	—	15	100
Controls 94	Positive	3	3 2	2	66 7	1	33 3	—	—	—	0
	Negative	83	88 3	56	67 5	7	8 5	20	24 0	—	0
	Doubtful	8	8 5	6	75 0	1	12 5	1	12 5	—	0

Reproduced by permission from *Annals of Internal Medicine* [38]
† Clinical picture suggested cat scratch fever Several were patients with abscesses or other pathological conditions (proved later)

value has been demonstrated Brand and Finkel [47] report some benefit to systemic symptoms from the use of chloramphenicol chlortetracycline and oxytetracycline but such compounds do not affect the course of the lymphadenitis Others [49, 50] report negative results with antibiotics

If it is true as some have suggested [2,47] that the cat is the only vector of the disease the control procedure becomes clear On the other hand if cats are merely mechanical vectors the solution of the problem of prevention is the discovery of the real source of the causal agent

And so it becomes clear that the playful kitten and the gentle companionable house cat have taken on a new role namely that of purveyors of an insidious and sometimes dangerous disease process Such news must come as a shock to many who look to the domestic feline as a symbol of homely comfort synonymous with the warmth and cheer of the hearthstone But who would not be disturbed when the last retreat in a troubled world is invaded by an unseen enemy and as if by a terrible twist of fate the entrance is facilitated by the second best friend of man?

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TABLE 1*

RESULTS OF COMPLEMENT FIXATION TESTS WITH LYGRANUM CF ANTIGEN SERA FROM PATIENTS AND CATS

No. sera (patients)	No. negative	Complement fixing titers (positives)					
		1:5	1:10	1:20	1:40	1:80	1:160
Single 20	10	4	1	1	?	1	1
Paired 2†	0	1 A x	—	—	—	—	—
		B x	—	—	—	—	—
		2 A x	x	x	—	—	—
		B x	x	x	x	x	—
Cats 4	4	—	—	—	—	—	—

* Reproduced by permission from *Annals of Internal Medicine*.[23]

† The two paired sera were obtained approximately two weeks apart. A = acute phase; B = convalescent phase. Both patients were ill several weeks prior to the obtaining of the acute specimen.

such results are not specific, and are negative in over 50 per cent of the cases. A positive CF test has less significance in mature and elderly individuals since members of these age groups normally carry such CF antibodies.

It was demonstrated by Foshay that a specific skin test antigen could be prepared from the exudate of the affected lymph node. Material is treated in a manner similar to that used in the preparation of the Frei antigen for lymphogranuloma diagnosis. Pus is collected in a sterile manner and diluted 1:1 with sterile saline or buffer. The material is heated to 60° C. for 2 hours. It is then stored for 24 hours in the refrigerator and reheated to 60° C. for an additional hour. Bacterial sterility tests are run and, with the advent of newer techniques, tests in tissue culture for the presence of cytopathogenic viral agents.

One tenth ml. of the antigen is injected intradermally in the forearm and the results of the tests are read 48 hours later, though some reactions are clear at 24 hours. An area of erythema with or without induration about the site of injection constitutes a positive reaction; however, areas of only 1 to 3 mm. in diameter are considered doubtful.

The efficacy of skin testing in the diagnosis of cat scratch fever has been considered by many workers (TABLE 2). The consensus is that it is of a high order of specificity, although some persons react without prior history of disease; such false positives are few.

It is known that ability to develop a positive reaction to the antigen may persist for many years. One case is reported in which the reaction was positive 28 years after clinical disease. Subsequent secondary subclinical infection, of course, may be a factor in the persistence of the reactive state.

Prevention and Therapy

No adequate measure for prevention by use of biological products is available. Many therapeutic agents have been used, but it is doubtful that real

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	Negative†	25	10.0	5	20.0	15	60.0	5	2.0	25	100
	Doubtful	15	6.0	15	100.0	—	—	—	—	15	100
Controls 94	Positive	3	3.2	2	66.7	1	33.3	—	—	—	0
	Negative	83	88.3	56	67.5	7	8.5	20	24.0	—	0
	Doubtful	8	8.5	6	75.0	1	12.5	1	12.5	—	0

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And so it becomes clear that the playful kitten and the gentle companionable house cat have taken on a new role, namely, that of purveyors of an insidious and sometimes dangerous disease process. Such news must come as a shock to many who look to the domestic feline as a symbol of homely comfort synonymous with the warmth and cheer of the hearthstone. But who would not be disturbed when the last retreat in a troubled world is invaded by an unseen enemy and, as if by a terrible twist of fate, the entrance is facilitated by the second best friend of man?

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Cats 4	4	—	—	—	—	—	—

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	Negative	83	88 3	56	67 5	7	8 5	20	24 0	—	0
	Doubtful	8	8 5	6	5 0	1	12 5	1	12 5	—	0

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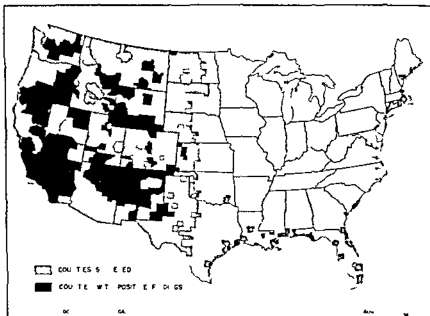


FIGURE 1 Animal plague in the United States from 190[illegible] through 19[illegible]0

Numerous species of wild rodents have been implicated in the epizootiology of plague ever since the first suggestive evidence of an epizootic in ground squirrels, *Citellus beecheyi*, was found in Contra Costa County, Calif., from 1903 to 1905 (Meyer, 1942b). When plague was conclusively demonstrated in ground squirrels by McCoy (1908) and by Wherry (1908), the attention of investigators was focused upon the larger colonial rodents. Thus Eskey and Haas (1940), in their classic study of plague in the western part of the country, indicated that the primary reservoirs of plague were ground squirrels, *Citellus* spp., wood rats, *Neotoma* spp., and prairie dogs, *Cynomys* spp. Occasional findings of plague in native mice, such as *Peromyscus* spp., mentioned by these and other authors, were considered by them to be unimportant accidental infections acquired by contact with ground squirrels or other important reservoirs.

Colonial rodents best served the purpose of the early plague surveys, which were conducted primarily to demonstrate the geographic extent of the disease. The adoption in 1936 of routine inoculation of pooled fleas in addition to tissue inoculations by Eskey and Haas (1940) increased the efficiency of surveys in delineating the rodent and flea reservoirs of plague. Consequently, tissue and flea pools from ground squirrels and prairie dogs constitute the major source of plague positive records thus far accumulated on wild rodent plague in this country.

In more recent years there has been a growing conviction among investigators of plague that rodents other than the ground squirrel and the large conspicuous

NEW KNOWLEDGE ON THE ECOLOGY OF SYLVATIC PLAGUE

By Leo Kartman, Frank M Prince Stuart F Quan, and Harold E Stark

San Francisco Field Station San Francisco Calif Communicable Disease Center U S Public Health Service

There is every reason to believe that an epidemic of plague is a self regulating phenomenon which is capable of description provided enough of its ecology is known Indeed it would remove a great deal of the fearsome unknown if one could forecast the peregrinations of plague which in man are merely accidental sequels to a broad rodent parasitism in his environment —Karl F Meyer (1942a)

Introduction

The history of plague is an eloquent example of changing concepts of investigation in which numerous hypotheses have been advanced, their limitations described their positive values extracted and their remaining skeletons buried in favor of new ideas Known in classical antiquity in the Middle Ages and in the pandemia of the latter Nineteenth and early Twentieth centuries plague has been studied primarily as an epidemic disease causing widespread mortality in human populations Until comparatively recently plague was thought to be fundamentally a disease of domestic rats in which rat fleas were the major vectors By 1928 it was clear that sylvatic or wild rodent plague represented an independent epizootiologic entity and since that time it has been recognized that sylvatic plague may transfer to domestic rodents under certain conditions (Pollitzer 1954)

Detailed historical and ecologic views and reviews of plague in the western United States have been written by Meyer (1942a and 1942b) while the works of Pollitzer (1954) and Macchiavello (1954) document the vast world literature The world distribution of sylvatic plague has been summarized by Garnham (1949) Sylvatic plague is now known to be established in wild rodents and their fleas in the western region of the United States and in western Canada Recently the presence of wild rodent plague was shown in Mexico with the isolation of the organism from prairie dogs in the northern state of Coahuila (Varela and Vasquez 1954) FIGURE 1 shows the distribution of plague in animals as a result of extensive surveys especially from 1936 to 1950 conducted by the United States Public Health Service and by certain states The predominance of wild rodent over rat borne plague is shown in FIGURE 2 Wild rodent plague foci have been found in 131 counties of 15 western states in surveys from the Pacific Coast to the 100th meridian an area comprising about 40 per cent of the continental United States The evidence shows that the disease in wild rodents forms a vast enzootic reservoir as a potential threat to humans Pollitzer (1954) lists 91 plague cases and 57 deaths in 7 western states during the period 1908 to 1951 as having been contracted from wild rodent sources In June 1956 a fatal human case occurred in Ventura County Calif this was apparently contracted from ground squirrel fleas FIGURE 3 shows the distribution of human plague cases resulting from contact with wild rodents with the exception of the case shown in Michigan which is a laboratory infection

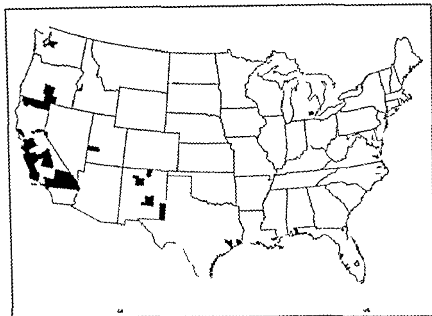


Figure 3 United States counties in which human plague has occurred

mice *Microtus* spp and *Dipodomys* spp are resistant to experimental plague infection

It is important to note that some of these small rodents are generally more closely associated with domestic rats *Rattus* spp in the immediate environs of human habitations than are the ground squirrels and other colonial species The potential danger is obvious and in the words of Meyer (1955) The increase in the population of California has given rise to two situations that must be taken into account The building of suburbs ultimately makes a habitat unsuitable for maintaining appreciable wild rodent populations but there is at least an initial period of joint tenancy by people and wild and commensal rodents—a condition theoretically ideal for the propagation of plague The wilderness recreational areas of California have been crowded with more people every year This too has increased the opportunity for close contact between man and reservoir animals and ectoparasites

It is known that native field mice and domestic rats coexist and live in newly suburbanized areas around refuse disposal sites on ranches in recreational areas and even in city vacant lots If plague infective fleas from field mice transfer to rats causing plague then infective rat fleas are introduced into the human environment The dangers inherent in the close association of wild rodents and domestic rats were recognized over 40 years ago by McCoy (1910) Eskey (1938) found the wild rodent flea *Malaraeus telchinum* on Norway rats in San Francisco From an analysis of data based on 5785 fleas

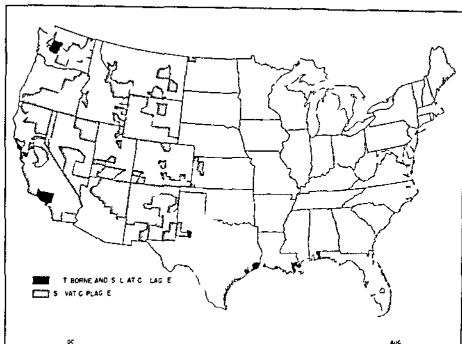


FIGURE 2 Rat borne and sylvatic plague in the United States from 1900 through 1949

species may be more usual reservoirs of *Pasteurella pestis* The colonial rodents are like man perhaps only occasionally included in plague outbreaks initiated by epizootics among native field mice (Meyer 1955) In California since 1908 investigations have shown that plague in wild rodents was enzootic in three fourths of the state and by 1952 the following 10 genera of native wild rodents and 2 genera of lagomorphs and/or their fleas were implicated with plague *Citellus Neotoma Peromyscus Eutamias Tamiasciurus Glaucomys Marmota Thomomys Microtus Reithrodontomys Lepus* and *Sylvilagus* (Jones and Street 1956) A perusal of the California records shows that plague positive fleas have been taken from the small native field mice on numerous occasions For instance during 1943 in Monterey County meadow mice (*Microtus californicus*) white footed mice (*Peromyscus* spp) and their fleas were discovered to be infected with plague in an area where ground squirrels were thought to have been virtually eradicated by intensive control operations (Jones and Street 1956)

Epizootiologic studies in various parts of the world have indicated that the mammalian plague reservoirs of primary importance are not necessarily the highly susceptible conspicuous species Some native mice that are relatively plague resistant may constitute the more usual reservoirs for the persistence of plague as has been found in Africa (Heisch *et al* 1953 and Davis 1953) Preliminary findings in California (Quan and Kartman 1956) and in New Mexico (Holdenried and Quan 1956) suggest that certain strains of native field

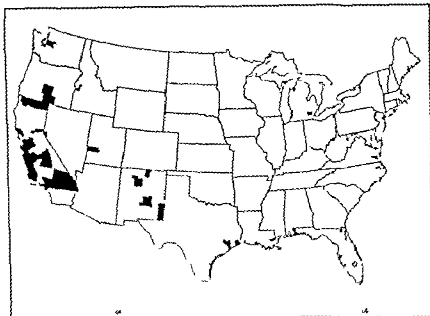


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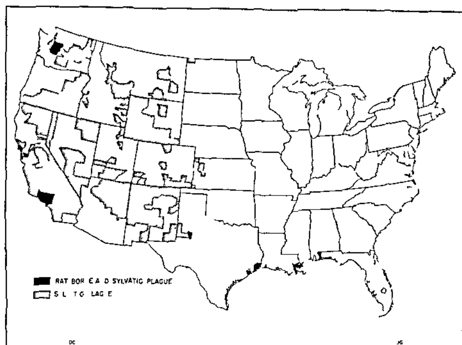


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suggest what the present position is in regard to various ecologic problems and to control Each item will be discussed under appropriate headings

The history of sanitary legislation has been a reflection of the evolution of theories and knowledge of the epidemiology and control of the relevant communicable diseases —H S Gear and L Deutschman (1956)

Plague in the United States and the International Sanitary Regulations

The fascinating history of quarantine practices has been told recently by Hirst (1953) and briefly summarized by Gear and Deutschman (1956) Plague *is one of the so-called quarantinable diseases against which legislation has* been primarily directed from the time of the early sanitary conventions to the present International Sanitary Regulations (World Health Organization 1951) In modern practice quarantine regulations on plague deal with rodent control on ships and in ports the isolation of human contacts or actual plague cases and disinsectization of ships and aircraft Article 51 of the International Sanitary Regulations specifically states Each state shall employ all means in its power to diminish the danger from the spread of plague by rodents and their ectoparasites Its health administration shall keep itself constantly informed by systematic collection and regular examination of rodents and their ectoparasites of the conditions of any local area especially any port or airport infected or suspected of being infected by rodent plague

The theoretical basis for these rules is rapidly becoming obsolete *The extent* and severity of plague epidemics has significantly declined throughout the world and it should not be long before most ports will feel secure as the result of the reduction of rat populations and because of adequate medical facilities Nevertheless there is a growing cognizance that even when the danger from rats has been lowered the danger of carryover from *wild* rodent sources is still a fact to be considered seriously Thus the World Health Organization Expert Committee on Plague (1953) stated Taking into consideration that the relationship between wild rodent and domestic rodent plague has not been fully elucidated it was further recommended that countries in which plague is still endemic should undertake systematic studies on *ecologic* lines to establish the comparative importance of wild and domestic rodent species in the maintenance of the plague reservoir and that particular attention should be given to a comprehensive investigation of the vector ability and survival of plague infected fleas under different conditions of temperature and humidity both in the laboratory and in the field

In the above quotation we have italicized the word ecologic since this is the area of investigation least understood in modern concepts of the plague As will be seen in other sections of this paper plague ecology is now the chief concern of the San Francisco Field Station even though routine surveillance of formerly plague infected port cities is still maintained It has been suggested (Gear and Deutschman 1956) that plague in rural rodents need not be given very much consideration in international traffic However it appears obvious that as long as wild rodent plague represents a possible source of the infection to urban areas it must be considered in the philosophy of quarantine We

from 4188 domestic rats caught in 13 western states Prince (1943) showed that these rats harbored 15 different species of wild rodent fleas Meyer and Holdenried (1949) established the existence of plague in rats *Rattus r rattus* and *R norvegicus* and in ground squirrels *Citellus beecheyi* on a ranch in Ventura County Calif Rat fleas were not found on the squirrels but 25 per cent of the fleas taken from the rats were squirrel fleas *Diamanus montanus* and *Hoplopsyllus anomalus* These authors concluded that plague was transferred to the rats by these wild rodent ectoparasites

Although this introduction has merely presented a few of the more important facts concerning plague in the western region of the United States it is sufficient to indicate that studies of plague in this country have only scratched the surface of the formidable epizootiological complex represented by the phrase "wild rodent plague" There is a definite need for intensive ecologic studies to elucidate the host ectoparasite disease relationships in plague foci especially those found near human populations The hazard of plague to the public health at present cannot be accurately evaluated therefore and only ill defined recommendations can be made for safeguarding humans against the enzootic plague reservoir It is generally agreed that while it is impracticable to attempt the control of wild rodent plague on a wide scale natural plague foci should not be permitted to exist where they threaten human populations The potentially dangerous nature of natural focal localizations or "nidi" of diseases transmissible to man have been described in detail by Pavlovsky (1955) It is now somewhat vaguely suggested that plague free zones should be maintained by the elimination of foci around populated areas or places temporarily inhabited by man This concept is confronted at the outset by the paucity of techniques for determining rapidly the presence or absence of plague in a given area Furthermore the currently meager knowledge of the ecology of plague precludes a sound judgment of such basic considerations as the weakest link in the infection chain the seasons of prevalence and quiescence practical protective zones and the types of habitats reservoirs and wild domestic rodent contacts that may harbor plague locally and present a threat to humans

Pioneering work on plague ecology in California has been done by a group of scientists formerly at the University of California Hooper Foundation Berkeley Calif (1939 to 1946), under the direction of K F Meyer These studies did much to show the direction in which modern concepts of plague ecology must travel More recently the Communicable Disease Center (CDC) of the Public Health Service established a field station at Santa Fe N M (Holdenried 1951) where studies of plague ecology in small native rodents were carried out during a three year period During the last six years the San Francisco Field Station of the CDC has initiated field and laboratory studies on the ecology and control of plague in the San Francisco Bay area (Kartman Miles and Prince 1958 Miles Kinney and Stark 1957) and on the control of enzootic plague in Hawaii (Kartman and Lonergan 1955a and 1955b) It would be beyond the scope of the present paper to discuss sylvatic plague on a world wide basis We shall therefore attempt to give an account of our own investigations in the western United States and to

families in the United States All these rodents are diurnal and fairly large and are therefore more frequently observed by man *Cynomys* noted for its colonial habits is most frequently involved in the spectacular die offs in which whole prairie dog towns covering hundreds of acres have been virtually extirpated Among other rodents cricetine and microtine species have yielded plague positive fleas and tissues on a number of occasions but no spectacular die offs have been observed as in the case of prairie dogs Most of these animals are nocturnal and all are small and inconspicuous thus permitting far less chance for gross observation Eskey and Haas (1940) reported plague in epizootic form among *Neotoma* the pack rats Field workers noticed many deserted nests of this animal When opened these nests revealed dead animals Although plague was not isolated from their tissues the organism was found in fleas removed from the nests

Recently new views have developed as to which rodents constitute the basic plague reservoir Multimammate mice in South Africa are considered almost entirely responsible for transfers of wild rodent plague to *Rattus* (Davis 1953) Heisch *et al* (1953) in East Africa and Baltazard *et al* (1952) in Persia consider resistant rodents to be basic plague reservoirs Holdenried and Quan (1956) found that various rodents in New Mexico were resistant to experimental infections of plague Data presented below show a correlation of plague resistance and the actual occurrence of plague in some small rodent populations in the San Francisco Bay area of California

One of the first objectives in ecologic studies conducted in San Francisco was to determine areas of association between domestic rats and wild rodents and to detect exchange of fleas between these wild rodents and domestic rats Hooper (1944) recorded 43 species and subspecies of native rodents from 9 ecologic associations in the San Francisco Bay area In our studies 12 native rodents were found in association with 3 species of domestic rodents in 4 ecologic associations as follows (1) *Salicornia* marsh association (2) bunch grass meadow association (3) sage coyote brush association and (4) riparian association Of the 12 rodents at least 4 were found to commingle with rats in significant numbers These were *Microtus californicus* (2 subspecies) *Reithrodontomys megalotis* (1 subspecies) *Peromyscus maniculatus* (1 subspecies) and *Citellus beecheyi* (2 subspecies) All ecologic associations encountered were variously modified by suburbanization industrialization or farming The trap sites may be characterized as follows dumps hog farms dairies parks highway developments factories feed mills and food packing plants All of these were located close to the bay or in mountains yet unclaimed by suburban developments Five mammal groups all belonging to orders other than Rodentia were encountered Only one group *Sorex* (3 species) was taken in significant numbers All of the individuals in the remaining 4 groups constituted about 1 per cent of the total capture Table 1 presents a list of the most important mammals trapped in descending order of frequency of capture The data presented are from a study by Miles *et al* (1957) and from subsequent and more detailed studies made in some of the areas where plague was found The 7 mammals listed constituted virtually 100 per cent of the total captures The remaining 10 mammals not shown were captured

have isolated plague infected rat fleas from the port of Tacoma Wash on at least two occasions in recent years Surveys in that port have shown that microtine rodents live in close contact with domestic rats in vacant lots and other areas of the city Thus there exists the strong possibility until proved otherwise that rats had acquired plague from wild rodent sources Actual transfer of plague from native field mice to rats has been found in our studies in the San Francisco area (Kartman *et al* 1958) The fact that transfer of plague actually does take place under modern conditions of sanitation emphasizes the thin tissue of protection now afforded humans living in certain areas It would probably take only a slight breakdown in present conditions to allow an increase in plague to spread to domestic rats Under disaster or emergency conditions such a spread could be explosive With present rapid means of travel an infected person could move thousands of miles away from the sylvatic plague source before the onset of clinical symptoms

We can conclude only that the international danger of the spread of plague from wild to domestic reservoirs is at present not subject to clear definition More intensive ecologic studies are needed for an exact delimitation of wild rodent plague and for an intelligent assessment of its epidemic potential It would seem that until such knowledge is available the international sanitary code must give proper recognition to sylvatic plague as an international menace and not treat it merely as a disagreeable local phenomenon

> The ecology of wild rodent plague is most complex to unravel it is like following the different voices in a Bach fugue except that in plague the basic design is unknown —P C C Garnham (1949)

Some Ecologic Factors in Sylvatic Plague

Rodent Species Involved

It is recognized (Pollitzer 1954 Macchiavello 1954) that both mammals and fleas may serve either as reservoirs or vectors of plague The important factor in the occurrence and transmission of plague is not simply whether or not *the mammal or flea is a reservoir or a vector but the degree to which it* serves in either capacity and what species is involved Macchiavello (1954) lists 344 mammal and 2 bird species that have been reported from various parts of the world as being experimentally infected as naturally infected or from which plague infected fleas were removed The great majority of these mammals are rodents of which 67 species occur in the Nearctic region Tables presented by Pollitzer (1954) and by Macchiavello (1954) include certain rodents that were easily infected experimentally with plague and thus presumed to be of importance However as will be pointed out later some rodents that have been proved to be ecologically implicated with plague are quite resistant when tested in the laboratory These rodents may actually be of greater importance to the perpetuation of the disease though they do not exhibit spectacular die off rates

Rodents of the family Sciuridae are of great importance in plague They have been involved in contacts with humans under sylvatic conditions and have transmitted plague directly to man more often than have other rodent

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TABLE 1

WILD MAMMALS TRAPPED FROM THE SAN FRANCISCO BAY REGION OF CALIFORNIA IN 13 AREAS WHERE DOMESTIC RODENTS WERE PRESENT

Species collected	Number trapped	Per cent of total	Flea index (all species)	Approx. distances between captures (ft)
Microtus californicus	2442	60.0	5.3	<100
Peromyscus maniculatus	528	12.0	2.7	>100
Reithrodontomys megalotis	454	11.0	0.3	>100
Rattus norvegicus	273	7.0	2.3	>500
Mus musculus	237	6.0	0.2	>100
Sorex sp	110	3.0	0.4	not known
Citellus beecheyi	23	0.5	38.2	>500

* A total of 37 individuals of 10 additional mammal species were trapped, or about 0.9 per cent of all animals taken.

incidentally. Either they were not in close association with feral rats or they were present in very limited numbers.

It will be noted that the ground squirrel *Citellus beecheyi* has the greatest number of fleas per host. However, squirrel fleas are usually highly host specific, thus tending to minimize the importance of commingling with domestic rodents. *Microtus* accounted for the greatest number of fleas taken from all areas, *Peromyscus* and *Rattus* each having lesser concentrations of fleas. *Sorex*, *Reithrodontomys*, and *Mus* had very low concentrations of fleas.

The first conclusive evidence of plague transfer from wild rodents to rats was reported by Meyer and Holdenried (1949) in an ecologic study of a plague epizootic in a feral colony of rats closely associated with *C. beecheyi*. The authors indicated that squirrel fleas were involved in the transfer. The work by Miles *et al.* (1957) suggested that in suburban areas there may be frequent flea interchange between smaller rodent species and rats. *Microtus californicus* appeared to be of greatest importance as regards flea exchange in the areas studied. In areas where the concentrations of both rats and small rodents were fairly equal, the index of fleas on alternative hosts was considerably higher than in areas where one or the other host predominated.

Blair (1940) has shown that the home range of small rodents such as *Microtus* probably is quite restricted, most individuals spending an entire lifetime in an area smaller than one acre. Grids of live traps were used in our more detailed studies of certain areas. The interval that seemed to have the best results was 30 feet. Our findings indicated that the ranges of the small rodents were quite limited. The range of *Peromyscus* extended relatively farther than that of other native rodents. Hayne (1950) discussed the relative merits of grid trapping and the subject will not be pursued further.

In the San Francisco Bay area *Microtus* was definitely confined to meadow bunch grass association and rapidly gave way to other species as the ecologic association changed. In one area, rats and mice (*Mus*) were located only in *Salicornia* association bordering the bay, while *Microtus* and *Reithrodontomys* were in an adjoining grassy area. The line of demarcation was both definite and constant. In most other areas where domestic rats were encountered

Microtus and other wild rodents were repeatedly observed to coexist in the same niche however the rats dominated their particular home territory

Rats usually build burrows underground and within trash piles if these are available and they usually persist in a given area until environmental pressures force them to move Kartman *et al* (1958) noted for a brief period the presence of rats along a small stream a short distance from a hog farm Following a poisoning campaign at the environs of the hog farm by the owners and by county health authorities the rats disappeared from the stream location perhaps returning to the hog farm where the pressure from the previously larger population was eliminated *Microtus* usually were observed to build their nests either above ground in a protected place or about 12 inches beneath the ground somewhere in their intricate tunnel systems It may be that *Microtus* like other rodents builds two types of nests a resting nest built by all individuals and a breeding nest built only by females for rearing young It appears therefore that the location of *Microtus* nests is a product of the type of area In loose sandy soils the nests were invariably located under ground in heavy claylike or soggy soils they were built within dense clumps of vegetation (usually in *Juncus* sp) or at the base of shrubs (*Baccharis*) *Peromyscus* and *Reithrodontomys* nests were difficult to find they seemed to be located mainly above ground in shrubs The predominant rodent of the sage coyote brush association was *Peromyscus* The heaviest concentration of fleas occurred on *Microtus* this may be due primarily to the nest building habits of this rodent

The unexplained complexity of sylvatic plague ecology is exemplified in the following account of recent observations in one of our study areas near San Francisco During November 1956 it was evident that a spectacular disappearance of *Microtus* was taking place this apparently had its beginning in mid October Six rodents were taken from the dwindling population of the area and autopsied to determine the possible presence of disease Nothing was detected in the blood in histological sections and animal inoculations of brain liver spleen heart or lungs of the rodents or from flea inoculations of laboratory mice The *Microtus* population remained low until the beginning of April when trapping was extended to include all areas plague positive in previous years in northern San Mateo County (south of San Francisco) The first trappings produced plague positive findings simultaneously in 5 areas and in 2 more weeks a total of 7 positive areas had been located After the first plague finding new trapping grids were established in a section of the area that looked most promising for a study of the physical extent of wild rodent plague The predominant types of flora and fauna in this area were grasses and few forbs the California vole *Microtus californicus* and the fleas *Malaraeus telchinum* *Hystrichopsylla linsdalei* and *Atyphloceras multidentatus* *M californicus* constituted more than 90 per cent of the rodent hosts

From April 16 through July 3 the capture of *Microtus* per trap day rose from 6 to more than 60 Plague was detected each week during that period by inoculations of flea pools into laboratory mice by plate culture of individual fleas and by cultures from occasional *Microtus* found dead in the field No generally adverse effect on the *Microtus* population was noted *Microtus*

nests were removed at intervals and some *Microtus* were taken for tissue and serologic tests Fleas from 38 nests were examined for plague and 5 contained *P pestis* Eight *Microtus* were found with plague positive tissues The study showed that plague was not limited to a single ecologic niche, but was found in various types of biotic communities in which *Microtus* and its fleas were present

Three significant findings were made during this study

(1) Plague occurred after the decimation of a ' stable ' *Microtus* population No plague had been detected from October 1955 to April 1957 at which time it became evident in a *Microtus* population that had dropped below the level of associated rodent species that are usually considerably less numerous than the predominant vole population

(2) The *Microtus* population increased rapidly at a time when plague was being found in about 1 per cent of the fleas taken from the animals and from their nests and in the tissues of some of the rodents

(3) In view of the fact that neither plague nor any other disease agent was detected in the *Microtus* during the October April period there is at present no explanation for the extreme reduction of the population at a time when *Microtus* populations in other areas appeared stable

Other reports of reductions in vole populations have been attributed to extreme competition for food However in the present case there was an ample supply of food and nesting material at all times It is notable that plague was found in all 7 areas within a span of 3 weeks without any obvious warning of its presence Thus it became apparent that plague appeared at a level readily demonstrable in the laboratory but without creating a noticeable effect on the *Microtus* or other small wild rodents over a wide area As noted above plague appeared after a decline and during a rise in the *Microtus* population Heisch (1956) notes in his studies of sylvatic plague in Africa that outbreaks usually coincided with an increase in the wild rodents However unlike the *Microtus* in our studies the African rodents were reported to decline suddenly at times due to plague On the basis of present knowledge it would be difficult to speculate on this mechanism of epizootic plague and impossible to explain it We feel that the highly resistant *Microtus* population and its fleas constitutes the *modus vivendi* of wild rodent plague in this area However the mode of operation of the complex is still obscure

The ecologic information gathered thus far points to the importance of the occurrence of small rodents as related to the perpetuation of plague in an area Furthermore a given area is able to support greater numbers of small individuals than of large ones The smaller native rodents may thus be a basic link in the plague chain serving as reservoirs of the organism during periods of apparent quiescence and supplying the source of infected fleas that may transfer the disease to domestic rats and then to man

Rodent Susceptibility

The rodent hosts of *Pasteurella pestis* not only vary in epizootiological importance but also show marked differences in ecology Some of the factors underlying these variations are evident and known one is the experimental

susceptibility to infection Correlation of human infection with sylvatic plague in Transbaikalia and Mongolia was well recognized in 1850 according to references cited by *Pollitzer* (1954) At that time the susceptible Siberian marmot was implicated in plague epidemics A hypothesis pointing to wild rodents as the reservoir of sylvatic plague was proposed in 1910 by McCoy (quoted by Pollitzer 1954 p 265) After finding insusceptible ground *squirrels in plague foci in California he concluded that this partially resistant race of rodents will if not vigorously attacked perpetuate the disease for many* years Later from an extensive series of investigations on California ground squirrels Meyer (1942a) explained that at the apparent termination of the epizootics both *susceptible and resistant rats or squirrels may survive and inter* breed furnishing sufficient hosts to maintain the infection in a smoldering non readily recognizable state Recently Baltazard and his associates (1952) were satisfied that in Iranian Kurdistan the *Meriones* subspecies which were somewhat *resistant to infection with P pestis were an important reservoir* of the disease Some investigators (Meyer 1942b Tinker and Kalabukhov 1934) found that young squirrels were highly susceptible to laboratory plague infections

That rodent species somewhat *resistant to infection are important reservoirs* of sylvatic plague has been confirmed in our laboratory and we believe that the highly prevalent and resistant meadow vole *Microtus californicus* is a significant factor in the sylvatic plague reservoir in the San Francisco Bay area However it *is not yet known what this resistance is or how it fits into the* ecologic puzzle Also it is not known what part is played by other rodents some of which are highly susceptible but may have an increased resistance during estivation or hibernation some species have been found infected with *P pestis* and thus *may serve as temporary reservoirs* The relative suscepti bility to the plague bacillus has been determined for only a few of these rodent species

Recent studies were directed toward elucidating the susceptibility to *P pestis infection of all the species of rodents within relatively small areas* In Santa Fe N M (Holdenried and Quan 1956) about 400 individuals of 21 wild rodent species were compared with a highly susceptible strain of white laboratory mice by intracutaneous inoculation of 0 05 ml of aqueous 1 per *cent peptone* dilutions of a virulent *strain (Alexander) isolated earlier from* a human case in New Mexico (Link 1950b) In the San Francisco Bay area (Quan unpublished data) about 1100 animals of 5 wild rodent and 2 feral domestic species were identically compared but in San Francisco guinea pigs and white laboratory rats (Sprague Dawley strain) were used as *controls* in addition to white mice The intracutaneous route of inoculation was em ployed in these tests because the investigators felt that this route best simulated the bite of the flea *a natural method of plague infection*

Although the rodent species tested in New Mexico and in California were different in the main one fact was outstanding in the results both areas were inhabited by rodent species varying from highly susceptible to completely *resistant individuals* (TABLE 2) Holdenried and Quan (1956) have divided these into 4 groups as follows

TABLE 2

APPROXIMATE PLAGUE LD_{50}s OF SOME OF THE SPECIES OF RODENTS TESTED IN NEW MEXICO AND CALIFORNIA

Rodent species	Santa Fe N M		San Francisco Calif	
	No tested	Approx LD	No tested	Approx LD
Citellus variegatus	9	$>10^4$	0	—
Dipodomys ordii	22	R	0	—
D spectabilis	13	R	0	—
Microtus californicus	0	—	461	$>10^4$ $<10^6$
M longicaudus	15	$>10^4$	0	—
Neotoma albigula	32	<10	0	—
Onychomys leucogaster	10	$>10^4$	0	—
Peromyscus boylii	11	$<10^4$	0	—
P leucopus	49	<10	0	—
P maniculatus	53	$<10^2$	273	$>10^4$
P truei	74	<10	2	$>10^4$
Reithrodontomys megalotis	8	<10	148	<10
Thomomys talpoides	9	$>10^4$	0	—

* R = refractory to intracutaneous inoculations of about 10^6 *Pasteurella pestis*

Highly susceptible Approximately the same susceptibility as white laboratory mice having an LD_{50} of less than 10 virulent plague bacilli by intracutaneous inoculation for example *Reithrodontomys megalotis* in both New Mexico and California

Susceptible to slightly resistant The majority died from 1 to 1000 mouse LD_{50}s (mouse LD_{50} about 10 organisms) for example *Peromyscus maniculatus rufinus P boylii rowleyi* of New Mexico and *Rattus norvegicus* of California

Moderately resistant to resistant Some deaths with doses greater than 1000 mouse LD_{50}s for example *Microtus longicaudus mordax* and *Onychomys leucogaster pallescens* of New Mexico and *Microtus californicus* and *Peromyscus maniculatus* of California

Highly resistant to refractory No deaths with doses of about 1 million virulent *Pasteurella pestis* for example *Dipodomys ordii medius* in New Mexico The susceptibility of a rodent species could be estimated and classified in one of the above groups by intracutaneous inoculation of as few as 2 or 3 animals each with undiluted 10^{-3} and 10^{-6} dilutions of a 24 hour second transfer brain heart infusion broth culture of a virulent *P pestis* strain grown at 28 C Such a culture usually has 100 million to 300 million organisms per milliliter

An observation of importance made in Santa Fe and in San Francisco was the lack of noticeable die-off of rodents during plague epizootics When *P pestis* was found in New Mexico (Holdenried and Morlan 1955) no decrease in rodents per trap per night was detectable In the San Francisco Bay area epizootic (Kartman *et al* 1958) *Microtus* were primarily involved but here again there was no decrease in the *Microtus* count

The finding of more resistant ground squirrels in California plague foci than in plague free areas (Meyer 1942b) is well known In an attempt to check this variance at Santa Fe four species of rodents (*Neotoma albigula Peromyscus truei P leucopus* and *P maniculatus*) from areas with and without plague

TABLE 3

SUSCEPTIBILITY OF *MICROTUS CALIFORNICUS* FROM VARIOUS LOCATIONS NEAR SAN FRANCISCO, CALIF., TO INTRACUTANEOUS INOCULATION OF ABOUT ONE MILLION PLAGUE ORGANISMS, *PASTEURELLA PESTIS*

Host locality	Plague found	Number used			Per cent dying		
		Males	Females	Total	Males	Females	Total
San Francisco game refuge	+	72	78	150	12.5	12.8	12.6
Central Hog Farm	+	40	40	80	12.5	10.3	11.2
Colma Creek	?	19	31	50	15.8	9.7	17.0
Assoc. Hog Farm	?	11	19	30	27.2	25.1	23.4
South San Francisco	—	49	54	103	6.5	64.8	66.0

were compared. No difference was observed in the degree of susceptibility of the comparative groups of these rodent species. It is yet impossible to explain precisely these contradictory results. One reason may be the difference in species employed. Another possible explanation is that plague-free or plague-positive areas in Santa Fe could not be delineated exactly. The importance of geographic separation is indicated (TABLE 3) by the finding that in a well-isolated area in south San Francisco, where *Pasteurella pestis* has not been found, *Microtus* were susceptible to plague infection (Quan and Kartman, 1956). It should also be pointed out that in the California plague foci studied by Meyer (1942b) and by the present authors, *P. pestis* has been found many times and probably has a continuous existence, while the status of plague in northern New Mexico has not been determined.

In spite of reports of varying degrees of infection in *Meriones* (Lobanov and Federov, 1938) and in susliks (Gaiski, 1926) during different times of the year, Davis (1948) stated that "rodent epizootics, whether in wild or domestic species, do not show any marked seasonal incidence." Our data (TABLE 4) substantiate this observation, since no significant seasonal differences were noted in tests with three species of wild rodents at different times of the year. On the other hand, studies at the Hooper Foundation (Meyer, unpublished data) have shown that guinea pigs were more resistant to experimental plague from October to March than at other times. Evidence of hibernation as a factor in the prolongation or modification of *P. pestis* infections in rodents also needs further elucidation (see Pollitzer, 1954 for review). The general importance and specific effects of season on wild rodent plague is but poorly understood at present.

The factors of age and sex of host also need further study. Meyer (1942b) reported that young ground squirrels were most susceptible, adult males less so, and adult females least so. Pollitzer (1954) felt that the higher susceptibility in young wild rodents was more apparent than real, since it was possible that the higher resistance of adults was due to hibernation. Also, in contrast to the above, Tinker and Kalabukhov (1934) found adult males more resistant than adult females. Our own results (TABLE 5) with *Microtus* do not show any marked differences either in the age (based on body length) or in the sex of the

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are presented by Pollitzer (1954) and by Macchiavello (1954) who list locality hosts and epidemiologic findings There are certain important ecologic relationships that affect a flea s importance in connection with plague and these relationships are observable mainly in the field Some of these are (1) the natural plague infection rate of different flea species (2) flea host relationships (3) flea nest relationships (4) abundance of different species seasonally on hosts and in nests (5) geographic location of fleas and finally (6) the natural systematic relationships of flea complexes or individual species to plague vector capacity

On a world wide basis the following are some of the fleas thought to be important in plague (Costa Lima and Hathaway 1946 Jellison and Good 1942 Jellison *et al* 1953 Macchiavello 1954 Pollitzer 1954) *Dinopsyllus ellobius Neopsylla setosa Leptopsylla segnis Diamanus montanus Nosopsyllus fasciatus Orchopeas sexdentatus Polygenis gwyni Ctenocephalides felis Echidnophaga gallinacea* and in the genera *Thrassis* (particularly *acamantis* and *bacchi*) *Oropsylla* (particularly *idahoensis* and *silantiewi*) *Opisocrostis Malaraeus Monopsyllus* (particularly *anisus* and *wagneri*) and *Xenopsylla* (particularly *cheopis astia* and *brasiliensis*) Recently Blanc (1956) has restated the case for *Pulex irritans* as an important vector in certain regions This list contains but a fraction of the number reported in laboratory and field investigations concerned with plague It would probably be cut in half and then again enlarged by the addition of many different fleas if the actual ecologic importance of each flea to plague could be considered and compared Kartman (1957) has proposed a guiding model in this direction by suggesting a method that combines field and laboratory findings to provide a single comparative rating Although detailed data are required for such information they are justified for those flea species implicated in wild rodent plague ecology

During recent work in the ecologically and geographically limited area around San Francisco certain species were studied quite exhaustively in relation to plague Some of the findings are presented in detail by Miles *et al* (1957) dealing with a preliminary study of 13 areas in the vicinity of San Francisco Bay and by Stark *et al* (1958) reporting an intensive ecologic study of one area where plague was found TABLE 6 (compiled from data of these studies) shows flea relationships with the hosts from which they were taken It should be noted that these data present figures on the percentage of occurrence of a given flea species (Miles *et al* 1957) This was defined as the index of a given flea on a particular host as the percentage of all flea host indices of that flea The resultant numerical values compensate for the disproportionate numbers of hosts obtained thereby modifying the significance of the usual index (mean number of fleas per host) and in addition may be somewhat diagnostic of the host preference of the flea A simple listing of host flea records (as for instance *Microtus californicus* with ten-odd species of fleas) only serves to clutter current literature without providing significant information It is felt that the number of species of fleas on a given host is directly proportionate to the frequency of capture of the host involved and this must weight the literature considerably Of concern to plague ecology studies are the fleas that appear in large numbers Vertical and horizontal

TABLE 4

MORTALITY FROM PLAGUE OF 3 SPECIES OF RODENTS TESTED AT DIFFERENT MONTHS OF THE YEAR IN SAN FRANCISCO, CALIF.

Month, year of test	Number dying/number tested and death rate among indicated species							
	Microtus californicus				*Peromyscus maniculatus*		*Reithrodontomys megalotis*	
	moderately resistant		susceptible		moderately resistant		susceptible	
February 1955	8/44	0.18	—		2/17	0.12	3/4	0.75
January 1957	2/31	0.06	6/7	0.86	5/25	0.20	8/11	0.73
March 1957	11/43	0.25	—		9/49	0.18	3/9	0.33
April 1955	5/24	0.21	—		1/9	0.11	7/10	0.70
May 1954, 1955	5/19	0.26	17/28	0.61	1/34	0.03	20/36	0.56
June 1955, 1956	14/42	0.33	30/39	0.72	—		8/13	0.67
July 1956	3/19	0.16	4/4	1.00	1/10	0.10	4/12	0.33
August 1955	2/8	0.25	10/21	0.48	7/32	0.22	3/7	0.43
October 1954	5/42	0.12	—		4/46	0.09	22/25	0.88
November 1954	2/17	0.12	—		2/9	0.22	2/8	0.25
December 1956	23/68	0.34	4/5	0.80	2/42	0.05	6/13	0.46
Total	80/357	0.22	71/104	0.68	34/273	0.12	86/148	0.56

All rodents received 0.05 ml. intracutaneously containing varying numbers of *Pasteurella pestis*.

TABLE 5

SUSCEPTIBILITY TO EXPERIMENTAL PLAGUE OF *MICROTUS CALIFORNICUS* ACCORDING TO SEX AND AGE

Sex	Body length (mm.) from tip of nose to tip of tail														
	105 to 130			131 to 150			151 to 170			171 to 190			All lengths		
	No. used	No. dying	Per cent dying	No. used	No. dying	Per cent dying	No. used	No. dying	Per cent dying	No. used	No. dying	Per cent dying	No. used	No. dying	Per cent dying
Male	13	4	12.2	68	20	12.1	90	20	10.6	27	10	34.4	198	54	12.2
Female	20	5	15.3	98	19	11.5	99	31	16.4	2	1	3.4	219	56	13.4
Totals	33	9	27.2	166	39	23.4	189	51	26.9	29	11	37.9	417	110	26.3

* As determined by body length.

rodent. However, some of the groups tested had too few individuals to furnish conclusive results.

Flea Species Involved

A priori every flea is a potential vector; whether a particular species of flea is actually a significant vector is another matter (Jordan, 1948). Tables of flea species implicated with plague in nature and in the laboratory

are presented by Pollitzer (1954) and by Macchiavello (1954) who list locality, hosts and epidemiologic findings. There are certain important ecologic relationships that affect a flea's importance in connection with plague and these relationships are observable mainly in the field. Some of these are (1) the natural plague infection rate of different flea species, (2) flea-host relationships, (3) flea-nest relationships, (4) abundance of different species seasonally on hosts and in nests, (5) geographic location of fleas, and finally (6) the natural systematic relationships of flea complexes or individual species to plague vector capacity.

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TABLE 6

HOST RELATIONSHIPS OF FLEAS COLLECTED FROM MAMMALS IN 13 AREAS IN THE SAN FRANCISCO BAY AREA

Host species	*Sorex sp.*		*Citellus beecheyi*		*Reithrodontomys megalotis*		*Peromyscus maniculatus*		*Microtus californicus*		*Rattus norvegicus*		*Mus musculus*	
Number examined	110		23		454		5 3		244		273		2 3	
Flea species	Flea index	Percent occurrence	Flea index	Percent occurrence	Flea index	Percent occurrence	Flea index	Percent occurrence	Flea index	Percent occurrence	Flea index	Percent occurrence	Flea index	Percent occurrence
Hystrichopsylla linsdalei					0.02	3	0.08	14	0.43	74	0.05	9.0		
Atyphloceras m. multidentatus					0.08	14	0.09	16	0.27	47	0.14	24.0		
Corrodopsylla c. obtusata	0.14	97					*	3						
Catallagia wymani	0.10	8	0.04	3	0.07	6	0.38	31	0.48	38	0.16	13.0	0.01	1.0
Leptopsylla segnis							0.01	3	0.01	3	0.25	76.0	0.06	18.0
Diamanus montanus			32.2	100							1.01	0.3		
Nosopsyllus fasciatus	0.01	1	0.09	6	0.03	2	0.07	5	0.07	5	1.10	76.0	0.07	5.0
Opisodasys k. nesiotus	0.04	2			0.05	3	1.60	92	0.03	2		0.3		0.3
Monopsyllus w. wagneri							0.08	64	0.01	8	0.03	24.0		4.0
Malaraeus telchinum	0.16	3	0.4	1	0.08	1	0.57	11	4 0	[illegible]4	0.48	9.0	0.07	1.0
Hoplopsyllus anomalus			5.8	100										
Xenopsylla cheopis										3	0.16	94.0		3.0

* Value less than 0.005; never more than 1 flea involved.

comparisons of the percentage of occurrence (TABLE 6) provide an indication of host specificity of the flea (or the degree of acceptability of the host) and numbers of flea species with high occurrences. The percentage of occurrence varied slightly when data from different areas were compared. There was, however, a definite limit to the range of the percentage of occurrence; for example, that of *Opisodasys nesiotus* on *Peromyscus* was always observed to be 90+.

Specific host-flea relationships in the San Francisco Bay region may now be discussed. Fleas such as *Ctenocephalides felis felis* and *Dactylopsylla ignota franciscana* were taken in low numbers, but since their normal hosts (that is, the domestic cat and the pocket gopher) were not considered for computations presented here, confusing values would be obtained on hosts that are presented. For this reason they are not included in TABLE 6.

Hystrichopsylla linsdalei. This flea was found in great concentration on *Microtus* in all areas studied and, from field and laboratory findings, it was considered to be the most important flea in wild rodent epizootics in the San Francisco Bay area. TABLE 7 shows it to have the highest percentage of plague positives in the field. In the laboratory this flea was found to have an experimental vector index of 0.02 as compared with 0.06 for *Xenopsylla cheopis* (Kartman and Prince 1956). Stark *et al.* (1958) present a vector potential computed from the vector index, field infection index, and

TABLE 7

NATURAL INFECTION RATES OF VARIOUS FLEA SPECIES DURING A PLAGUE EPIZOOTIC AMONG FIELD MICE MARCH 23 TO APRIL 30 1954

Collect week No	Infection rate of indicated fleas													
	Malaraeus telchinum		*Hystrichopsylla linsdalei*		*Atyphloceras multidentatus*		*Catallagia wymani*		*Opisodasys keeni*		Others*		All species	
	No	Per cent pos	No	Per cent pos	No	Per cent pos	No	Per cent pos	No	Per cent pos	No	Per cent pos	No	Per cent pos
1	316	10 1	42	23 8	26	0	26	3 8	12	25 0	3	0	425	10 8
2	421	5 7	61	9 8	30	3 3	45	0	1	0	0	—	55	5 6
3	257	4 2	41	14 6	21	0	25	0	9	0	0	—	353	4 8
4	190	4 4	28	7 1	17	5 8	30	0	1	0	0	—	256	4 3
5	132	0	28	10 7	16	0	19	0	10	0	1	0	206	1 5
6	251	0	47	2 1	36	2 7	53	0	13	0	4	0	404	0 5
Totals	1557	4 8	247	11 3	146	2 0	198	0 5	46	6 5	8	0	2202	5 0

* Mainly *Nosopsyllus fasciatus*

field prevalence index which was greater than that of any of the other fleas encountered This flea appeared less likely to transfer to other hosts such as rats than did some of the other flea species

Atyphloceras m multidentatus This species was fairly abundant and showed a field infection index below that of *Hystrichopsylla* and *Malaraeus* (TABLE 7) However these data were based on a six week period Studies carried on for a whole year with additional plague findings gave *A multidentatus* a higher rate than *Malaraeus*

Catallagia wymani In one study the number of individuals of this flea exceeded that of *H linsdalei* However this flea was restricted to a more limited type of environment than other species and was entirely absent in some areas where plague appeared The percentage of occurrence for *C wymani* indicated that this flea had the widest range of acceptable hosts therefore it may be of considerable importance in transfer between rodents The field infection rate was exceedingly low and laboratory findings (Eskey and Haas 1940) indicated that it was probably not a vector of importance

Diamanus montanus This flea has been found to be an efficient vector of plague (Eskey and Haas 1940 Wheeler and Douglas 1941 Burroughs 1947 Kartman and Prince 1956) Its high index on *Citellus* not only in the San Francisco Bay area but in all western states indicates that this flea is of importance in plague epizootics among the ground squirrels However its importance may be restricted because of its high degree of host specificity Even in areas where opportunity for transfer was afforded (for example to *Rattus* which is an acceptable host to many flea species) the percentage of occurrence and high index (TABLE 6) indicated a rather unusual host preference Nevertheless the work of Meyer and Holdenried (1949) shows that this species on occasion may transfer plague to domestic rats

Nosopsyllus fasciatus The northern rat flea is a fairly efficient experimental

vector of plague (Eskey and Haas 1940 Burroughs 1947), being approximately equal in this respect to *H linsdalei* In the areas studied in the vicinity of San Francisco its numbers were low, and it was not found to be infected in the plague areas where it occurred However, because of its wide range of accept able hosts as indicated by its percentage of occurrence it may be potentially of importance in any part of the San Francisco Bay area, where a build up of the rat population would increase the incidence of this flea In a recent survey of the city of San Francisco a general index of 1 5 X *fasciatus* per rat was evident However, the purpose of this survey was to avoid a general index and to seek out rather the index in relation to specific factors such as season habitat of the rat and section of the city Surprisingly high indices were found in some sections of the city which indicated that favorable conditions still persist in some areas for the propagation of these fleas and their rat hosts despite recent socioeconomic improvements

Opisodasys keeni nesiotus This flea was of some importance in the plague picture since it is a known experimental vector and because it had a high field infection rate (TABLE 7) However Stark *et al* (1958) reported a rather low vector potential due to its scarcity in the areas studied Like *D montanus* it appeared restricted by a strong host specificity It may be of importance to plague among small rodents whenever its principal host *Peromyscus* sp is present in fairly large numbers

Monopsyllus wagneri ophidius The very low index of this flea in the San Francisco Bay area was rather surprising since it is a major flea of several rodents over much of the United States This finding adds one more factor to the uniqueness of this section of Pacific Coast flora and fauna

Malaraeus telchinum This species was the most abundant flea in all areas studied Its principal host *Microtus* occurred in rather limited ecologic environments and the flea appeared to be restricted by the host s distribution In other parts of the United States *Peromyscus* is the principal host Populations of *Microtus* are always quite dense and therefore are conductive to a high flea population The range of acceptable hosts and the degree of host specificity of *M telchinum* were similar to those of *Hystrichopsylla linsdalei* Although laboratory findings indicated that these fleas are poor individual vectors at best our field studies revealed a fairly high infection rate This fact taken in conjunction with evidence of mass transmission in the laboratory (Burroughs 1944 1947) suggests that this species may be an important secondary vector in the field

Hoplopsyllus anomalus This flea was found to be naturally infected with plague in New Mexico (Holdenried and Morlan 1955) and is able to transmit plague (Eskey and Haas 1940) but is probably a poor vector (Wheeler and Douglas 1941) Like *Diamanus montanus* it was restricted to its host because of a high degree of specificity

The remaining fleas *Corrodopsylla curvata obtusata* and *Xenopsylla cheopis* were taken in very low numbers on their preferred hosts *Sorex* and *Rattus* respectively Whether or not these two species of fleas are of importance to sylvatic plague cannot be ascertained from present studies The occurrence of these two species in sylvan areas may be of significance X *cheopis* oc

curred in large numbers on urban rats in San Francisco being a very close second to *Nosopsyllus fasciatus* A recent survey of San Francisco rat fleas substantiated previous findings that *X cheopis* occurred more frequently under strictly urban conditions while the reverse was true for *N fasciatus*

In general there seemed to be very little correlation between the seasonal incidence of fleas on their hosts and the occurrence of plague *H linsdalei* had a uniform incidence on its preferred host *Microtus californicus* throughout the year The incidence of *Malaraeus telchinum* followed fairly closely that of its host *Microtus* as indicated by trapping results Increases in flea indices followed shortly behind the increase of host prevalence The peak of *M telchinum* occurred during and following the warmer and drier season of the bay region In contrast *Catallagia wymani* had its greatest incidence during winter and spring and its lowest following the drier season There were insufficient data to consider seasonal incidence for other fleas

The numbers of fleas on the host tell very little about what occurs in the host s nest where the adult fleas seem to spend a good deal of their time and on which the immature stages are completely dependent Bacot and Martin (1914) when writing up their remarkable discovery of blocking in fleas theorized on the effects of adverse climatic conditions on blocked fleas Other workers ascribed the trend of plague seasons to the influence of climatic conditions on fleas (Petrie 1929) However studies of these effects on fleas and plague have not progressed very far Hirst (1926) and Grubbs (1927) have postulated that even though an infection is present there is little danger of epidemic spread as long as the *Xenopsylla cheopis* index remains below 1 Stewart and Evans (1941) considered that the value of flea surveys in the accepted sense of collecting fleas from the rodents themselves might be enhanced by obtaining the composition of the flea population at the openings of wild rodent burrows Davis (1939) and Macchiavello (1948) have recommended that the total or absolute numbers of fleas should be determined for surveys by the examination of burrows and nests Consequently some workers have advocated the use of a formula in which the absolute flea index is obtained by dividing the total number of rats into the total number of fleas found on rodents and in their nests It must be emphasized here that ecologic studies of plague in the field go beyond the survey method Other types of data are needed especially in relation to the life of the flea vector in the host s nest For instance we have found that the optimum quantity of moisture in nesting material was between 50 and 70 per cent as indicated by numbers of fleas per nest In nature nests most frequently showed the lowest moisture range (10 to 30 per cent) and least frequently the highest range (70 to 90 per cent) *Catallagia wymani* flourished under relatively high moisture conditions while *Malaraeus telchinum* prevailed in drier nests This type of microenvironmental investigation may reveal important factors in seasonal plague and the vector potentials of fleas and lead to the development of better methods for flea and rodent control and the laboratory culture of wild rodent fleas

Finally let us consider briefly the importance of systematics Some of the material presented in this paper illustrates the need for exact taxonomic identification of fleas as has been repeatedly demonstrated in the past The

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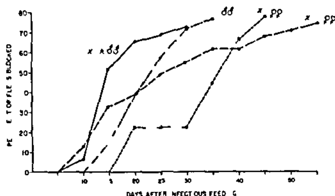


FIGURE 4 The cumulative blocking rate of fleas *Xenopsylla vexabilis hawaiiensis* and *X. cheopis* which were infected with *Pasteurella pestis* at various days after the infectious blood meal

More recently Kartman, Prince and Quan (1956) and Kartman and Prince (1956) have utilized an additional component, the blocking survival potential, to derive a vector index. It is based on a ratio of the mean day of death of fleas after blocking over the mean day of blocking after the infectious meal. This potential was suggested by the fact that the speed of blocking and the survival of the flea after blocking are critical factors affecting biological transmission of plague (FIGURES 4 and 5). The shorter the incubation period and the longer the survival after blocking, the greater the blocking survival potential. The vector index is the product of the vector efficiency and the blocking survival potential (or the product of the three potentials of Wheeler and Douglas and the blocking survival potential). As an example, TABLE 8 presents data from experimental tests with the native Hawaiian flea compared

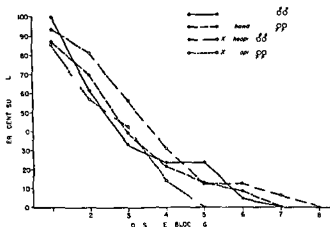


FIGURE 5 The longevity of fleas after blocking with *Pasteurella pestis*

Plague Commission in India (1904 to 1907) found that rat fleas were involved in transmission of plague, but was unable to determine that two prevalent but similar species (*Xenopsylla cheopis* and *X. astia*) were present in the endemic plague area. Fluctuations in the occurrence of human plague were not explained until these fleas had been identified and their nature determined. Of great significance was the fact that these fleas differed greatly in their ability to transmit and in their seasonal occurrence. Today confusion still exists as to the identity of *Thrassis howelli* and *T. stanfordi* and as to which species was actually involved in plague transmission experiments (reported in Eskey and Haas 1940; Macchiavello 1954; and Pollitzer 1954). In our field studies a flea of the genus *Hystrichopsylla* was encountered that had a higher natural infection rate and was a more efficient transmitter of plague than any other wild rodent flea. Exact taxonomic evaluation of this species showed that it had previously entered the literature under a misnomer (Burroughs 1944; Snodgrass 1946; Hubbard 1947). The flea was in fact a new species and observations were made on it for more than four years before its specific determination as *Hystrichopsylla linsdalei* was made in 1957 (Holland 1957).

*Vector Efficiency**

During the course of investigations of plague by many workers the concept of vector capacity of separate flea species became of paramount concern. When the importance of strict taxonomic classification of the flea species had been realized, the role of these species as vectors became a critical problem in studies of plague epidemiology. The classic work of the Plague Research Commission in India (1906 to 1915) constitutes the basic knowledge of the transmission of plague by the rat flea. The work of Bacot and Martin (1914) gave a conclusive demonstration of a mechanism of the infectious process in the flea and a mode of plague transmission by this insect.

Wheeler and Douglas (1941, 1945) attempted to assess the importance of several factors that, when combined, would give a more exact picture of the vector efficiency of individual fleas of a single species. They set up a mathematical model in which the factors could be combined in a product symbolic of plague vector capacity. These authors postulated three potentials for deriving vector efficiency: (1) the infection potential is the proportion of fleas becoming infected after feeding on an animal with plague septicemia; (2) the vector potential is the proportion of infected fleas that become infective or blocked (Kartman *et al.* 1956); and (3) the transmission potential is the mean number of transmissions accomplished by the infective fleas, each of which is fed daily upon a laboratory mouse. The vector efficiency is the product of these three potentials and represents the mean number of plague transmissions per flea. It should be noted, in the words of Wheeler and Douglas (1945), that ". . . the infection and vector potentials actually do not enter into the determination of vector efficiency; however, they are included to emphasize the fallacy of estimating the efficiency of a vector solely on the basis of the number that became infected or infective."

* For a more extended discussion see Kartman (1957).

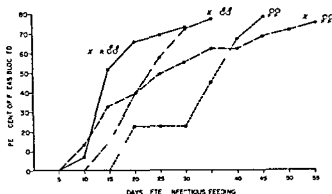


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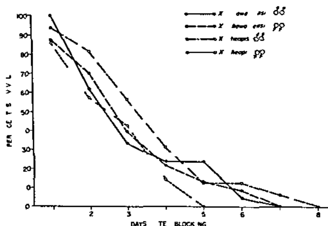


FIGURE 5 The longevity of fleas after blocking with *Pasteurella pestis*

TABLE 8
SUMMARY DATA FOR THE DETERMINATION OF PLAGUE VECTOR EFFICIENCY INDICES

Flea species	X. [illegible]			X. cheopis		
Sex	M	F	M + F	M	F	M + F
Number used	29	31	60	21	9	30
Number infected	23	28	51	21	8	29
Number blocked	21	23	44	16	[illegible]	23
Number of transmissions	9	11	20	12	9	21
Blocking survival ratio†	3 5/14 5	3 3/22 4	3 4/18 9	3 9/20 3	3 0/30 9	3 7/23 5
Infection potential	0 79	0 90	0 85	1 00	0 88	0 96
Blocking potential	0 91	0 82	0 86	0 76	0 87	0 79
Transmission potential	*0 4[illegible]*	*0 47*	*0 45*	0 75	*1 28*	0 91
Vector efficiency‡	0 30	0 35	0 33	0 5[illegible]	0 99	0 69
Blocking survival potential	0 24	0 14	0 17	0 18	0 09	0 15
Vector index	0 07	0 05	0 05	0 11	0 09	0 10

From Kartman *et al* 1956
† Mean day of death after blocking/mean day of blocking after infectious meal
‡ Vector efficiencies calculated according to the method of Wheeler and Douglas 1945

with the classic vector *Xenopsylla cheopis* (Kartman *et al* 1956) The vector indices suggest the differences in plague vector capacity of the two flea species under experimental conditions When atmospheric conditions plague strains and the fleas used are carefully controlled replications of this type of laboratory test will result in fairly consistent data

The central problem lies in the circumstance that the vector efficiency and the vector index as now used represent only a limited number of the factors actually involved in a total concept of vector capacity The actual need is for an index that will include pertinent data from field studies Few investigators would cavil at the suggestion that knowledge of the total ecology of a flea species would aid in evaluating its role as a vector of plague That is an ideal toward which workers with many arthropod borne diseases aim Nevertheless knowledge of detailed ecologic processes accumulates but slowly and the worker must deal with the level of known facts at any particular moment

Certain types of ecologic data are now fairly easy to obtain and they may be included in the evaluation of plague vector capacity The prevalence of various flea species or the flea index based on numbers of fleas per rodent is almost a routine observation in field studies Considerably less attention has been devoted to the flea index in rodent nests Nevertheless the most realistic flea index is one that combines data from both the rodent and its nest Furthermore no attempt has been made in plague ecology studies to obtain flea infection rates based upon bacteriological tests of individual fleas taken from rodents and their nests In recent studies we have paid particular attention to these factors and TABLES 7 and 9 are examples of data obtained With these factors in mind we have recently suggested and have used a model (Kartman 1957) for deriving categorical values that compare flea species in a hierarchy of relative importance in plague epizootiology This scheme at

TABLE 9

PLAGUE INFECTION IN FLEAS FROM NESTS AND FROM HOSTS (JANUARY 1955)

F l g	Flea species			
	M l aeu t l h m	*H l h p yll l d les*	*At pht m ltidentat*	*Cat ll g wym*
Mean fleas per nest	19 0	5 1	6 7	9 7
Mean fleas per host	4 7	0 6	0 4	0 6
Nest fleas per cent infected	4	33	15	0
Host fleas per cent infected	7	14	23	25

tempts to combine laboratory with field data in the following way flea vector potential = experimental vector index × field infection index × field prevalence index × 100

Although the numerical values thus derived represent a considerable amount of ecologic observation we must still agree with Girard (1955) that much obscurity still obtains in the problem of vector capacity Hypothetically it can be postulated that in any set of ecologic conditions governing the *epizootic wave or the enzootic trough the plague vector capacity of a flea* population is ultimately a function of the hospitality of the individual flea to the plague bacillus Thus the biochemical physiology of the flea plague relationship is an absolutely necessary avenue of investigation that has been neglected almost completely By the use of artificial flea feeding techniques (Kartman 1954) we have found for instance that certain strains of plague will grow normally and produce proventricular blockage in fleas when the bacteria are suspended in rat blood but will not multiply when the medium is rabbit blood Preliminary studies have shown that blockage in the flea may depend upon the number of bacteria ingested during its infectious feeding a definite threshold appear to exist Temperatures of about 20 to 22 C favor the growth of *Pasteurella pestis* in the flea whereas temperatures of 27 C and above are not only unfavorable to the ingested bacilli but also shorten the life of the flea itself The development of a proventricular block does not follow the same course in different flea species In *Xenopsylla cheopis* blockage is fairly rapid and a high percentage of individuals show initial development of blockage in the proventriculus On the other hand bacterial growth in most wild rodent fleas begins in the ventriculus and it may take as long as two months for proventricular blockage to occur under the same conditions

Although plague transmission by the individual blocked flea is an established mechanism in the natural history of the disease the phenomenon of mass transmission must be mentioned as another mechanism that has received insufficient attention As an example it is known from the studies of Burroughs (1944 1947) and of this laboratory that the wild rodent flea *Malaraeus telchinum* does not show proventricular blockage under conditions in which other species become blocked Thus plague transmission by individuals of this species almost never occurs in laboratory tests of vector capacity

If we compare this flea with another wild rodent species *Hystrichopsylla linsdalei* utilizing the concepts discussed above the following data (Kartman 1957) result

Hystrichopsylla linsdalei			*Malaraeus telchinum*		
Experimental vector index	=	0.07	Experimental vector index	=	0
Field infection index	=	0.11	Field infection index	=	0.04
Field prevalence index	=	0.08	Field prevalence index	=	0.43
Vector potential	=	0.02	Vector potential	=	0

Although these data appear significant they mean little, since we know that *M telchinum* is quite prevalent in the field and has a comparatively high plague infection rate during epizootic conditions (TABLES 7 and 9) A further indication of its importance is obtained when groups of many individuals are allowed to feed *en masse* on clean mice very soon after having fed on a mouse with terminal plague bacteremia In this case plague transmission is obtained quite consistently *M telchinum* is therefore considered an efficient mass transmitter presumably by mechanical transference of the plague bacillus on contaminated mouth parts

This mechanism of mass transmission is undoubtedly more important in plague ecology than has heretofore been assumed Our own studies suggest that mechanical transmission by a highly prevalent flea may be the means by which epizootics among wild rodents flare up once they have been initiated by primary vectors that transmit biologically via the blockage mechanism Experimental studies with *Xenopsylla cheopis* and mice (Quan *et al* 1953) showed that some of the fleas with at least partially blocked proventriculi were infective in the usual manner However the work also suggested that during the first few feedings after the infectious blood meal infection of the clean mice took place through the mechanism of plague contaminated mouth parts being washed by saliva Obviously the phenomenon of mass transmission must be taken into account in the ecology of wild rodent plague Devignat (1946) working with fleas in the Belgian Congo was the first to attempt a quantitative estimate of mass transmission Differences in vector capacity were determined by comparing the surface areas of the curves illustrating the mortality of flea infected mice with the surface area of a graph showing the mortality of mice infected with standard doses of *Pasteurella pestis* Future work is faced with the problem of integrating the concept of mass transmission with current hypotheses regarding the mechanisms involved in sylvatic plague

Transfer of Fleas Between Hosts

The literature contains numerous references to the so called exchange of fleas between rodent host species yet the evidence has been based primarily upon findings that certain flea species parasitize animals other than their normal or preferred hosts The concept of preferred hosts is in itself beset with pitfalls for the unwary observer for a considerable number of factors must be known before the parasite host relationship can be evaluated ade

quately With respect to fleas such factors as seasonal fluctuations nest populations and availability of specific hosts are of critical importance In personal correspondence H B Morlan has discussed the question of host preference in reference to his paper on mammal fleas in New Mexico (Morlan 1955) It is of interest here to quote from his remarks

Where data from extensive collections of all possible hosts at all seasons of the year are available the percentage of animals infested with one or more parasites and the mean number of parasites per examined animal are usually sufficient to indicate host preference In cases of inadequate collections host preference will remain unknown in spite of any additional manipula ion of insufficient data My paper on Santa Fe fleas provides an actual example where the mean number of *Opisocrostis hirsutus* from infested ground squirrels was 10 0 while the number from infested prairie dogs was 7 6 In this case only a few squirrels were collected mainly in the vicinity of a dog town and there was enough infestation of squirrels with the normal prairie dog flea (*O hirsutus*) to give a false impression of host preference The records merely indicate a specific instance of an unusual potential means for the spread of disease from prairie dogs to ground squirrels I am presently inclined to classify the hosts of an ectoparasite as normal secondary and accidental The normal host is that animal which has been demonstrated to be the most commonly encountered associate A secondary host is one that is frequently and consistently associated with a particular ectoparasite There may be more than one secondary host An accidental host is one that is infrequently and inconsistently found associated with a parasite

Morlan s remarks have been quoted at length because it is important to make clear cut distinctions in host preferences when evaluating the problem of flea transfer between host pecies In plague epizootiology it is of the ut most significance to determine the preferred host especially in cases where certain rodent pecies are known to be the prime reservoirs of plague Thus in our studies the native meadow vole *Microtus californicus* is thought to be the primary plague reservoir and this host in most instances is parasitized most frequently and in greatest numbers with those flea species showing the highest plague infection rates This relationship is true of the rodent nests as well as of the rodents and any transfer of plague to other wild rodent species or to domestic rats would suggest that the disease had originated in *Microtus* and its fleas

It should also be noted that except in clear-cut instances the finding of a wild rodent flea on a domestic rat or the presence of a flea on a wild rodent other than its normal host does not imply transfer of the fleas between hosts Too little is known of the breeding capacities of wild rodent flea pe ies in the nests of various hosts In the laboratory we have been able to culture the *Microtus* flea *Malaraeus telchinum* on albino rats This suggests that *Rattus norvegicus* in nature could possibly support the breeding of *Malaraeus telchinum* in its burrow If this is true the finding of *M telchinum* on domestic rats would not necessarily imply transfer from *Microtus* even if populations of the latter were prevalent in the vicinity of the rats Recent studies in the city of Tacoma Wash showed that *Microtus* nests contained cultures

If we compare this flea with another wild rodent species *Hystrichopsylla linsdalei*, utilizing the concepts discussed above the following data (Kartman 1957) result

Hystrichopsylla linsdalei		*Malaraeus telchinum*	
Experimental vector index	= 0 02	Experimental vector index	= 0
Field infection index	= 0 11	Field infection index	= 0 04
Field prevalence index	= 0 08	Field prevalence index	= 0 43
Vector potential	= 0 02	Vector potential	= 0

Although these data appear significant they mean little since we know that *M telchinum* is quite prevalent in the field and has a comparatively high plague infection rate during epizootic conditions (TABLES 7 and 9) A further indication of its importance is obtained when groups of many individuals are allowed to feed *en masse* on clean mice very soon after having fed on a mouse with terminal plague bacteremia In this case plague transmission is obtained quite consistently *M telchinum* is therefore considered an efficient mass transmitter presumably by mechanical transference of the plague bacillus on contaminated mouth parts

This mechanism of mass transmission is undoubtedly more important in plague ecology than has heretofore been assumed Our own studies suggest that mechanical transmission by a highly prevalent flea may be the means by which epizootics among wild rodents flare up once they have been initiated by primary vectors that transmit biologically via the blockage mechanism Experimental studies with *Xenopsylla cheopis* and mice (Quan *et al* 1953) showed that some of the fleas with at least partially blocked proventriculi were infective in the usual manner However the work also suggested that during the first few feedings after the infectious blood meal infection of the clean mice took place through the mechanism of plague contaminated mouth parts being washed by saliva Obviously the phenomenon of mass transmission must be taken into account in the ecology of wild rodent plague Devignat (1946), working with fleas in the Belgian Congo was the first to attempt a quantitative estimate of mass transmission Differences in vector capacity were determined by comparing the surface areas of the curves illustrating the mortality of flea infected mice with the surface area of a graph showing the mortality of mice infected with standard doses of *Pasteurella pestis* Future work is faced with the problem of integrating the concept of mass transmission with current hypotheses regarding the mechanisms involved in sylvatic plague

Transfer of Fleas Between Hosts

The literature contains numerous references to the so called exchange of fleas between rodent host species yet the evidence has been based primarily upon findings that certain flea species parasitize animals other than their normal or preferred hosts The concept of preferred hosts is in itself beset with pitfalls for the unwary observer for a considerable number of factors must be known before the parasite host relationship can be evaluated ade

TABLE 10

TRANSFER OF Ce^{144} TAGGED WILD-RODENT FLEAS *MALARAEUS TELCHINUM* FROM THE FIELD VOLE *MICROTUS CALIFORNICUS* TO THE DOMESTIC RAT *RATTUS NORVEGICUS*

No fleas placed on *Microtus*	60
No transfers to rats while *Microtus* were alive†	3
No fleas taken from rats after *Microtus* were killed‡	8
No fleas taken from rats allowed in same area with live *Microtus*	9
No fleas found in rat nests	27
Total number of fleas accounted for from rats	4
Percentage of recovery of fleas from rats	18 3

From data of W W Hartwell (unpublished)
† Rats in contiguous area but prevented from mingling with *Microtus*
‡ Rats allowed into area with dead *Microtus*

Radioactive fleas were also recovered from *Microtus* nests and radioactive feces were observed which showed that the rodents had ingested some of the fleas In general a higher percentage of tagged female fleas was recovered

Tests with domestic rats showed that tagged fleas would transfer most readily from *Microtus* to *R norvegicus* when infested *Microtus* were killed and allowed to remain in the area or when rats were allowed to enter the *Microtus* area and compete with them TABLE 10 summarizes the pertinent data Some indication of the life span of Ce^{144} tagged fleas was given by the fact that 76 5 per cent of the fleas were recovered from rats and from their nests after 50 days (TABLE 11) In all the trials tagged fleas showed an initial

TABLE 11

TIMES OF RECOVERY OF Ce^{144} TAGGED FLEAS *MALARAEUS TELCHINUM* FROM RATS *R. RATTUS NORVEGICUS* WHEN THE FLEAS WERE ORIGINALLY PLACED ON THE WILD RODENT *MICROTUS CALIFORNICUS*

Time (days)	Number of flea transfers	Test condition
	Fleas combed from rats	
18 29 33 34	1 2 1 4	Rats allowed in area with dead *Microtus*
38 42	1 2	Rats isolated in area contiguous with *Microtus*
50 51 56	4 2 3	Rats allowed free access to area with live *Microtus*
	Fleas from rat nests	
56	27	Nests removed after all above conditions terminated

From data of W W Hartwell (unpublished)

of the rat flea *Nosopsyllus fasciatus* Thus the finding of this rat flea on trapped *Microtus* need not necessarily imply transfer of the fleas from rats

In the introduction we have already noted the observations of McCoy (1910) Eskey (1938) Prince (1943) and Meyer and Holdenried (1949) on the subject of flea transfer between wild and domestic rodents In addition Meyer (1942b) presented evidence of flea transfer and Pollitzer (1954) has summarized the pertinent literature on this subject Recent studies at this laboratory (Miles *et al* 1957) have revealed several instances of finding rat fleas on wild rodents and wild rodent fleas on commensal rats

In plague epidemiology the significance of flea transfer is twofold On the one hand enzootic plague foci and strictly wild rodent plague epizootics depend on the transmission of the organism by efficient vectors that transfer between individual hosts of the primary reservoir species On the other hand the spread of plague either from one wild rodent species to another or from a wild to a domestic rodent species depends on transfer of infected fleas interspecifically between hosts The existing evidence suggests that transfer to rats may be fairly rare since no cases of rat plague have been found as yet in the interior of the western states That this is not due to a lack of commensal rats was shown during a plague epizootic of rabbits in New Mexico where plague free rats were not only present but also entirely free of flea in festation (Link 1950a)

On the basis of present knowledge two problems of prime importance must be solved (1) direct observation of flea transfer between host species must be shown in order to substantiate the current hypothesis of plague spread based on circumstantial evidence and (2) quantitative data on the commingling of wild rodent and rat populations must be gathered to evaluate more ade quately the potential for plague spread Some data on the first of these prob lems is presented here Recently this laboratory in cooperation with the University of California developed a simple method for the radioactive tagging of fleas and other arthropods with cerium^{144} (Quan *et al* 1957) Fleas were successfully tagged by bathing them for less than 5 minutes in aqueous solutions of Ce^{144} Pr^{144} containing 10 μc /ml Tagging was accomplished by a stable combination of cerium with the exoskeleton a reaction that is at present still obscure The isotope Ce^{144} has a half life of 282 days and the beta parti cles from its short lived daughter isotope Pr^{144} (half life 17 5 minutes) can be detected readily with the usual Geiger Muller survey meter Thus the means were developed to evaluate the problem of flea transfer between hosts *in the field* W V Hartwell recently conducted at our laboratory a pilot *experiment* in simulated field plots this was done on a small scale as a prelim inary to actual field studies The rodents *M californicus* and *R norvegicus* and tagged fleas *Malaraeus telchinum* were used in these tests The experi ments established the fact that under the more or less normal conditions of the rodents existence the fleas transferred between individual *Microtus* In a total of 5 trials 111 tagged fleas were placed on different individuals with observations lasting 15 days on the average and ranging from 6 to 21 days A total of 99 actual flea transfers was observed or an average of 19 transfers per test and the percentage of tagged fleas accounted for ranged from 30 to 82

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No fleas taken from rats allowed in same area with live *Microtus*	9
No fleas found in rat nests	27
Total number of fleas accounted for from rats	47
Percentage of recovery of fleas from rats	78 3

From data of W V Hartwell (unpublished)
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‡ Rats allowed into area with dead *Microtus*

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TABLE 12

WILD RODENT FLEAS *MALARAEUS TELCHINUM* *HYSTRICHOPSYLLA LINSDALEI* *ATYPHLOCERAS MULTIDENTATUS* FOUND NATURALLY INFECTED WITH *PASTEURELLA PESTIS* BY BACTERIAL CULTIVATION OF INDIVIDUAL FLEAS AND BY ANIMAL INOCULATION OF FLEA POOLS

Collect	Fleas cultured individually				Flea pools inoculated						Total fleas pos‡	Percentage fleas pos‡
	No tested	No pos	Percentage pos	Efficiency of test (percentage)	No pools tested	No pools pos	Percentage pools pos	No fleas pos†	Percentage fleas pos†	Efficiency of test (percentage)		
A	447	40	9.0	87	88	9	10.0	45	10.0	98	46	10.3
B	357	13	3.6	77	91	10	11.0	10	2.8	59	17	4.8
C	254	8	3.1	73	82	10	12.0	10	3.9	91	11	4.3
Total	1058	61	5.8	83	261	29	11.0	65	6.2	88	74	7.0

* Some positive pools contained more than one plague positive flea

† Individual culture checked on fleas used in pools—based on number of fleas shown in second column to the left

‡ Assuming that no positive fleas were missed by combination of both methods and assuming that only one positive flea was present in a positive pool from fleas found negative by culture

tion of the two methods revealed all plague positive fleas the culture method showed from 73 to 87 per cent of the positives and averaged 79 per cent the inoculation method showed from 59 to 98 per cent of the positives and averaged 83 per cent Thus the efficiency of detecting plague in the fleas by a combination of the two methods was 96 per cent (on the average 3 naturally infected fleas were not detected when 74 plague positive fleas were collected)

If the fleas had been processed only by the individual culture method 61 of 74 positives or 83 per cent would have been detected During these tests which will be reported in greater detail elsewhere it was felt that the efficiency of individual flea culture would be higher if trituration were accomplished by a tissue homogenizer rather than by manual crushing with a roughened glass rod in a test tube If the fleas had been processed solely by the animal inoculation method 65 positives out of 74 or 88 per cent would have been found In general a high level of efficiency can be expected with the animal inoculation method which is of value mainly in plague surveillance or when it is necessary to test for the presence of plague and to determine its extent on a rough scale The individual culture of fleas not only reveals the presence of plague efficiently but also provides a natural plague infection rate in different flea species Thus the latter method though tedious and time consuming is valuable in *more detailed ecologic research on wild rodent* plague and has provided types of data hitherto unknown

> The total absence of plague from the inhabitants of California for the past eight years could lead to the erroneous conclusion that control measures applied in this state for the past 50 years have completely eradicated the disease It is wiser to [illegible] the present level as a pl[illegible] t au[illegible] f unknown length and to re[illegible]st the temptation to draw conclusions about the causes —Karl F Meyer (1955)

average count of 6 2 to 8 5 $\times 10^2$ counts/flea/min and after 56 days, 3 6 to 5 1 $\times 10^2$ counts/flea/min

These preliminary experiments are not conclusive but they suggest that a wild rodent flea *Malaraeus telchinum* will readily transfer between individuals of its normal host species whereas transfer to domestic rats may take place only under unusual circumstances Nevertheless the transfer of wild rodent fleas to rats from dead wild rodent hosts is of special significance when applied to the problem of plague spread from sylvatic to domestic environments The fact that *M telchinum* will transfer to rats illustrates the most probable method by which domestic rats acquired plague during an epizootic among *Microtus* and other small field rodents (Kartman *et al* 1958) Undoubtedly, other important wild rodent flea vectors of plague will also transfer to rats

Detection of Plague in the Field

Winter and Moody (1957) have announced the adoption of Coons fluorescent antibody technique for rapid identification of *Pasteurella pestis* They observed that individual cells in a smear were brightly stained in bubo exudate and blood collected from a human case four days after onset of the clinical infection The method certainly appears to hold great promise for the detection of plague in the field especially of plague in flea specimens

Currently detection of plague in nature still depends on general epizootiologic observations and on inoculation of guinea pigs or mice with pools of rodent tissues and fleas In all probability die offs of larger colonial rodents still occur, and they may be due to plague However in themselves die offs may be difficult to locate especially those of the smaller native field mice Furthermore when plague resistant rodents are present die offs may not occur at all Thus in plague surveillance workers still rely chiefly on collecting fleas and inoculating susceptible laboratory animals with pools of them

Eskey and Haas (1940) rediscovered and popularized inoculation with flea pools for the detection of wild rodent plague foci Inoculation with flea pools for *Pasteurella pestis* has the following advantages (1) fleas may be plentiful even when rodents are relatively scarce (2) flea pools are less dangerous to handle than rodent tissues (3) the specimens are less likely to contain gross contamination than are tissues and (4) the fleas do not deteriorate as readily as tissues Flea pool inoculation tests however are not suitable for determination of the extent or the intensity of plague infection in a given rodent population It is rather surprising that in spite of its long and extensive usage the flea pool method has never been evaluated critically with regard to its efficiency as a method for detecting plague in naturally infected fleas

In recent studies by Quan and his co workers (unpublished) on wild rodent plague among native field rodents such as *Microtus* and *Peromyscus* the flea pool inoculation method was compared with the bacteriological culture of individual fleas The fleas were first individually crushed and triturated and then streaked on a blood agar plate The remaining portions of individual flea triturates were then pooled and used to inoculate laboratory mice The comparative results obtained by these two methods and an evaluation of their relative efficiencies are shown in TABLE 12 Assuming that the combina

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C ll ti	11 lt 1 1 1 lly				Fl pool oc lat d						T tal fl po ‡	P tag flea pos ‡
	t t d	po	P tag po	Effi y f t t (r t g)	pool t t d	pool p	P tag pool pos	fl p †	P tag fl po †	Effi y of t t (p t g)		
A	447	40	9 0	87	88	9	10 0	45	10 0	98	46	10 3
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Totals	10 8	61	5 8	83	261	29	11 0	65	6 2	88	74	7 0

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average count of 6.2 to 8.5 × 10 counts/flea/min and after 56 days 3.6 to 5.1 × 10 counts/flea/min.

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through the mechanism of flea exchange discussed in a preceding section Recent studies at this station have shown the importance of small native field mice (genera *Microtus Peromyscus* and *Reithrodontomys*) not only as a source of plague but also as a reservoir of infected fleas that may transfer to domestic rats The most important fact is that enzootic foci of plague are found near suburban developments farms and in the environs of cities and that wild rodents commingle with rats in such areas as well as in city garbage dumps small towns and camps The increase in suburban populations and the extension of human habitations into rural areas is probably the most significant factor in the present wild rodent plague problem Our studies in northern San Mateo County just beyond the San Francisco limits have shown that plague epizootics occur among the native field rodents and that plague actually transfers to domestic rats (Kartman *et al* 1958) Thus in the problem of wild rodent plague control close attention must be given to situations in which plague foci in native field rodents are found in areas where commensal rats occur adjacent to human habitations and human activity

It is undoubtedly true that under usual conditions in this country the sanitary practices that have evolved and that are continually being improved constitute effective barriers to the transfer of plague from wild to domestic rodents and thence to man Furthermore unlike the situation in other regions of the world there is no evidence here that the domestic rat constitutes an independent reservoir of plague infection In Madagascar for instance the periodic resurgence of localized murine plague foci has been noted without any evidence that rodents other than the rat are the primary source of the disease (Girard 1940) From the standpoint of public health the critical factor in our western region is the maintenance of effective barriers between the wild rodent reservoirs of plague and the domestic rats On the other hand the normal sanitary barriers could conceivably be severed in times of natural disasters such as floods and earthquakes and also during national emergencies especially in case of devastation due to war The latter may seem a highly remote possibility yet in the face of ever growing threats of international atomic conflict we must be prepared with the knowledge requisite for dealing with plague and other vector borne diseases Thus resources for public health must include not only a cognizance of wild rodent plague ecology but also an awareness of the most effective means for the detection diagnosis treatment and control of plague

Diagnosis and Treatment of Plague Cases

Today prognosis of plague is excellent provided the disease is diagnosed sufficiently early Rapidity of diagnosis however still depends heavily on both epidemiologic intelligence and sensitive rapid laboratory methods for detecting *Pasteurella pestis* and its antibodies Fortunately advances have been made along all these fronts It has been shown recently that the plague organism in human blood exudates and post mortem tissues is subject to rapid identification by use of fluorescent antibody techniques (Winter and Moody 1957) The detection of plague antibodies by the hemagglutination procedure (Chen 1952) has also proved useful (McCrumb *et al* 1953)

Public Health Aspects of Sylvatic Plague

In the introduction we have noted the large area in which evidence of wild rodent plague has been found in the western United States In contrast to this it must be pointed out that the number of human infections derived directly from wild rodent foci has been of a low order TABLE 13 which is modified after Pollitzer (1954) summarizes the incidence of human plague infection contracted from wild rodents or their fleas

The reason for the low level of human plague directly associated with wild rodents is not difficult to assess Foci of wild rodent plague are generally situated in sparsely populated and isolated rural districts where wild rodent fleas do not have the opportunity of biting man Even when humans do enter areas harboring wild rodent plague various biological factors intervene to prevent infection A person would have to be present during an epizootic that in most cases appears quite transitory He would have to be bitten by an infective (or blocked) wild rodent flea or simultaneously by a number of wild rodent fleas that had very recently fed on a septicemic animal The chances against this are great when the natural infection rate of fleas is taken into account and when the factor of host preference is considered Furthermore in a large flea population the number of blocked individuals would probably be very small and of those infective fleas present that actually did bite very few would be likely to accomplish transmission That plague transmission to humans by wild rodent fleas does occur is shown by TABLE 13 but according to the World Health Organization Expert Committee on Plague (1953) The unexpected occurrence of human plague in insufficiently explored areas draws attention to the need for an exact delimitation of wild rodent plague We might add that the occurrence of sylvatic plague near human habitations makes the ecology of wild rodent plague a paramount subject for investigation

The real danger from wild rodent plague occurs when wild and domestic rodents coexist in areas close to human habitation It is here that the possibility of plague transfer from wild to commensal rodents may take place

TABLE 13
INCIDENCE OF PLAGUE INFECTION ORIGINATING IN SYLVATIC FOCI

	Year in which plague was first found in		Human plague 1908 to 1956	
State	Wild rodents	Humans	Cases	Deaths
California	1908*	1908	81	53
Oregon	1935	1934	1	1
Utah	1936	1936	1	0
Nevada	1936	1937	1	0
Idaho	1936	1940	1	1
New Mexico	1938	1949	6	3
Arizona	1938	1950	1	0
		Totals	92	58

* Plague suspected in ground squirrels in 1903

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Today prognosis of plague is excellent provided the disease is diagnosed sufficiently early Rapidity of diagnosis however still depends heavily on both epidemiologic intelligence and sensitive rapid laboratory methods for detecting *Pasteurella pestis* and its antibodies Fortunately advances have been made along all these fronts It has been shown recently that the plague organism in human blood exudates and post mortem tissues is subject to rapid identification by use of fluorescent antibody techniques (Winter and Moody 1957) The detection of plague antibodies by the hemagglutination procedure (Chen 1952) has also proved useful (McCrumb *et al* 1953)

In the effective treatment of plague the evaluations made by Meyer and his co workers (1952) still represent the most modern concepts of therapy Smadel and his associates (1955) have added their experiences from the handling of pneumonic cases in Madagascar Specific plague therapy calls for a judicious administration of antibiotics and, when necessary the use of antiserum The antibiotics of choice are streptomycin chloramphenicol and the tetracyclines 'Sulfa drugs especially sulfadiazine and sulfamerazine are very useful both in treatment and for prophylaxis (Quan *et al* 1955) Vaccination is a logical procedure under certain conditions

Although further general discussion of plague therapy is beyond the scope of this paper, the fatal outcome in spite of effective treatment, of the 1956 bubonic plague case in California deserves comment This case was of sylvatic origin, it was diagnosed early and treated so intensely that no bacilli could be isolated at autopsy after but three days of therapy The death of the patient indicates that therapy still presents problems The patient (Sackacs) was a retired Chief Petty Officer in his middle forties He had been in good health and there was no reason to suppose that he was unusually susceptible or sensitive to plague Quan and Meyer (unpublished) have investigated the virulence and chemotherapy of the Sackacs strain of *P pestis*, a succinct report of the findings follows

From the bacteriological point of view the plague bacilli isolated from the bubo exudate of Sackacs were undifferentiated from any other virulent *Pasteurella pestis* strains Although the content of the fraction I protein (antigenic portion) of this California strain was not studied that of its fraction II content (toxic portion) was extracted and compared with an Indian strain (195/P) Both strains were equally toxic When compared with the Alexander and 195/P strains for growth in broth containing various concentrations of streptomycin aureomycin or chloramphenicol no differences were found in the sensitivity of the three strains of these antibiotics *in vitro* (TABLE 14) When mice were infected intracutaneously with about 500 bacilli of the above three strains and given therapy at 48 hours after infection the success of the antibiotics against the Sackacs strain was similar to that against infection by the

TABLE 14

GROWTH OF *PASTEURELLA PESTIS* IN BROTH CONTAINING ANTIBIOTICS

P t ll p t t	St pt my	A eomy	Chl mph l
	(t t on (µg/ml) t h b t y t t d y		
Alexander	4 0	1 0	2 0
Sackacs	4 0	1 0	2 0
195/P	4 0	1 0	2 0
	(t atso (µg ml) b t d l d y		
Alexander	8 0	32	4 0
Sackacs	8 0	32	4 0
195/P	8 0	32	4 0

* Broth contained 10^{6} microorganisms per ml

TABLE 15
SURVIVAL RATE OF MICE INOCULATED WITH *PASTEURELLA PESTIS* AND TREATED WITH ANTIBIOTICS

P. pestis strain inoculated	Antibiotic used in therapy			
	Streptomycin	Aureomycin	Chloramphenicol	None (control)
	Number survived/number treated			
Alexander	20/20	6/20	14/20	0/11
Sackacs	16/20	5/21	12/20	0/11
195/I	13/ 0	3/20	15/20	0/11

Infection was produced intracutaneously by injection of 0.05 ml containing about 500 *Pasteurella pestis*. The course of therapy was 3 days beginning 48 hours after infection. The daily dose was 100 µg I.I. followed in 8 hours by 200 µg subcut.

other two strains (TABLE 15). Although in some tests the Sackacs strain appeared to kill mice slightly more rapidly than did the other two strains, the difference was not significant. These investigations suggested that in the light of present knowledge the Sackacs strain was undifferentiated bacteriologically and chemotherapeutically from the New Mexico and the Indian strains.

The therapeutics may be a major factor in the fatal outcome of the California case. It was reported that in this particular case heroic dosages of all the effective plague medicinals were administered and that the patient was sterilized of *P. pestis*. Although this has not been heretofore observed in man, its possibility was indicated when Quan and his co-workers (1947) sterilized mice of plague bacilli by the use of streptomycin (Meyer *et al.* 1952). Nevertheless, it was not known what effect, if any, such sterilization might have on the host animal.

We know that experiments in the chemotherapy of plague with laboratory mice and guinea pigs show that the use of increasing dosages of effective drugs or combinations of drugs up to toxic dosages have elevated survival rates (TABLE 16). However, as may be seen from TABLE 16, even the intensive therapy was short of the sterilizing range of *P. pestis in vivo*. Thus, to test for sterilizing effect a level higher than intensive therapy, heroic therapy, was formulated from the pattern of the clinical treatment used in the California case. In that case sulfisoxazole, which is far less effective than sulfadiazine in experimental plague therapy (Quan *et al.* 1955), and penicillin, which is ineffective against *in vivo P. pestis* (Meyer *et al.* 1952), were used with streptomycin, chloramphenicol and oxytetracycline. In the heroic therapy sulfadiazine was substituted for sulfisoxazole but penicillin was included. All the drugs were adjusted for treating mice every six hours. The various dosages of the therapies are shown in TABLE 17. The moderate and heroic therapies were given to mice that had been infected intraperitoneally at a time when between 20 and 40 per cent of the animals were dead.

The results (TABLE 18) indicate a clear trend: excessive dosage of drugs given in the heroic therapy was less effective and saved fewer mice than did the moderate dosage. It should be noted that these are the results of but

TABLE 16
CHEMOTHERAPY OF MICE INOCULATED INTRACUTANEOUSLY WITH SACKACS AND ALEXANDER STRAINS OF *PASTEURELLA PESTIS*

Therapy	Initial time of therapy					
	36-hour infection		51-hour infection		66-hour infection	
	Alexander	Sackacs	Alexander	Sackacs	Alexander	Sackacs
	(percentage of recovery)					
Sulfadiazine	58	73	42	45	—	—
Sulfa and streptomycin	83	77	67	45	33	27
Intensive†	100	100	83	91	67	59

* Twenty two mice per group of Sackacs 12 mice per group of Alexander
† Intensive therapy consisted of a combination of sulfadiazine streptomycin terramycin and chloramphenicol

one experiment and are not conclusive K. F. Meyer has commented that these results may have been due to a mechanism analogous to that in the Herxheimer reaction Furthermore, it is possible that this strain of *P pestis* is more readily lysed *in vivo* when under antibiotic treatment than are other strains If these results are confirmed they suggest a new type of toxic effect of antibiotics in addition to those already known

Civilization alone has destroyed the plague in Europe it alone will destroy it in the East —Aubert Roche (quoted by H H Scott 1942)

Sylvatic Plague Control

In any discussion of the control of an arthropod transmitted disease of man cognizance must be given to the effects of human social development upon its rise or fall Hence the above quotation We cannot enter here into a dissertation on philosophies of history but it should be mentioned that certain men prominent in their field—Ronald Ross Angelo Celli Hans Zinsser and others—have promulgated a monistic theory that assumes that disease makes history Although we cannot deny that malaria typhus yellow fever and plague have often been strong influences against social progress we are inclined to accept the view of such scholars as Ackerknecht (1945) who discussed malaria in particular that the waves of these diseases can be more logically explained by historical events In his famous Milroy Lectures on plague Liston (1924) cited the second agrarian revolution in England during the Eighteenth Century as the unrecognized beginning in the conquest of plague in that country He discussed in detail the influences of social reforms sanitary measures and the prosperity associated with the period of high farming On the other hand Hirst (1953) explained this same decline in plague on the basis of a change in the rat and rat flea species without investigating the causal factors influencing reductions in black rat and *Xenopsylla cheopis* populations Very recently it was observed by Fox (1956) that typhus in San Juan Puerto Rico showed a 100 per cent decrease

TABLE 17
DOSAGES OF VARIOUS THERAPIES

Therapy	Dose/mouse (mg [illegible] ts)	Mg/kg	Known efficacy (per cent)
Mild			
Sulfadiazine	2 00	100	40
Moderate			
Streptomycin	0 20 0 25	10 12 5	
Sulfadiazine	2 00 1 00	100 50	60
Intensive			
Streptomycin	0 20	10	
Chloramphenicol	0 40	20	85
Terramycin	0 40	20	
Sulfadiazine	2 00	100	
Heroic			
Streptomycin	1 00	50	
Chloramphenicol	0 50	25	
Terramycin	0 50	25	
Sulfadiazine	1 00	50	
Penicillin	1 00	30	
Serum			
Antitoxic (intraperitoneal 0 5 cc of 1 to 5 dilution of 40 units/cc)			0

Average survival rate of mice infected intracutaneously with about 100 plague bacilli for 51 hours and then treated

TABLE 18
SEROTHERAPY AND CHEMOTHERAPY OF MICE INOCULATED INTRAPERITONEALLY WITH SACKACS STRAIN OF *PASTEURELLA PESTIS*

Therapy	Mortality [illegible] of the mice at time of initiation of therapy	
	20 per cent death	40 per cent death
	Survivors/number treated (per cent)	
Moderate	9/25 (36)	5/12 (41)
With serum	—	7/12 (58)
Heroic	5/25 (20)	4/11 (36)
With serum	10/25 (40)	5/11 (45)

Mice received about 5000 bacilli

at a time when absolutely no control operations were in effect as in the southern United States Thus Fox concluded that a reduced typhus rate is not necessarily proof of the efficacy of control measures Girard (1955) has evaluated the modern position on plague in the following nicely balanced terms The modern pandemia is declining This situation is legitimately attributed to the success of a prophylactic method the result of fruitful research but one must also include the contribution of natural circumstances in order to explain this regression in countries where the control has been too limited to be effective

All evidence points to a spectacular decline in human plague throughout the world (Gear and Deutschman 1956) Plague has apparently been conquered

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CHEMOTHERAPY OF MICE INOCULATED INTRACUTANEOUSLY WITH SACKACS AND ALEXANDER STRAINS OF *PASTEURELLA PESTIS*

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	36 hours after infection		51 hours after infection		66 hours after infection	
	Alexander	Sackacs	Alexander	Sackacs	Alexander	Sackacs
	(percentage of survivors)					
Sulfadiazine	58	73	42	45	—	—
Sulfa and streptomycin	83	77	67	45	33	27
Intensive†	100	100	83	91	67	59

* Twenty-two mice per group of Sackacs; 12 mice per group of Alexander.
† Intensive therapy consisted of a combination of sulfadiazine, streptomycin, terramycin, and chloramphenicol.

one experiment and are not conclusive. K. F. Meyer has commented that these results may have been due to a mechanism analogous to that in the Herxheimer reaction. Furthermore, it is possible that this strain of *P. pestis* is more readily lysed *in vivo* when under antibiotic treatment than are other strains. If these results are confirmed, they suggest a new type of toxic effect of antibiotics in addition to those already known.

> Civilization alone has destroyed the plague in Europe; it alone will destroy it in the East. —*Albert Roche (quoted by H. H. Scott, 1942)*

Sylvatic Plague Control

In any discussion of the control of an arthropod-transmitted disease of man, cognizance must be given to the effects of human social development upon its rise or fall. Hence the above quotation. We cannot enter here into a dissertation on philosophies of history, but it should be mentioned that certain men prominent in their field—Ronald Ross, Angelo Celli, Hans Zinsser, and others—have promulgated a monistic theory that assumes that disease makes history. Although we cannot deny that malaria, typhus, yellow fever, and plague have often been strong influences against social progress, we are inclined to accept the view of such scholars as Ackerknecht (1945) who discussed malaria in particular, that the waves of these diseases can be more logically explained by historical events. In his famous Milroy Lectures on plague, Liston (1924) cited the second agrarian revolution in England during the Eighteenth Century as the unrecognized beginning in the conquest of plague in that country. He discussed in detail the influences of social reforms, sanitary measures, and the prosperity associated with the period of high farming. On the other hand, Hirst (1953) explained this same decline in plague on the basis of a change in the rat and rat flea species without investigating the causal factors influencing reductions in black rat and *Xenopsylla cheopis* populations. Very recently it was observed by Fox (1956) that typhus in San Juan, Puerto Rico showed a 100 per cent decrease

small native field voles and meadow mice is difficult because their nests are hidden and do not occur in towns or community groupings as do those of the larger colonial rodents Furthermore wholesale dispersal of insecticidal dusts may not be safe in many areas near human habitation or human activity where the smaller rodents are found and where they present the chief potential for the spread of plague For these reasons flea control methods were developed in the plague endemic zone of Hawaii in which a DDT bait box was used (Kartman and Lonergan 1955a) The apparatus consisted of a covered bait station with DDT filled sacks hung at opposite entrances so that the rodent would receive a shower of DDT powder on entering and leaving the box The theory behind this method was that the rodent itself would become the vehicle by which the insecticide was dispersed to its nest and its mates In practice this method not only controlled those fleas on the rats but provided evidence suggesting that fleas in the nests also were affected Excavation of rat burrows showed a complete lack of fleas in the area of the DDT bait box whereas nests were 100 per cent infested in a check area and showed an average of about 25 fleas per nest Analysis of nesting material in one instance only showed DDT residues amounting to 0 72 mg In contrast to these results Wagle and Seal (1953) reported that DDT could be detected only in the first few inches of rat holes when the insecticide was applied by insufflation of the burrows Live fleas were found in 14 of 44 nests examined

Recent field trials have shown that a simplified insecticide bait box controls fleas on the heavily parasitized *Microtus californicus* as well as on other species of meadow mice both on the hosts and in their nests FIGURE 6 shows an end view of the bait box which consists of the following a floor board one half an inch thick 12 inches long and 8 inches wide a U shaped roof made by cutting in half lengthwise a lard tin 9½ inches in diameter and 12½ inches deep and a bait container held by small nails in the center of the floor board The bait boxes were placed at 50-foot intervals in a field grid 250 feet square A similar check area in the same field was established 150 feet from the test area After an initial prebaiting period with rolled oats about 80 gm of 5 per cent DDT powder was spread on the floor of each bait box Data on fleas in rodent nests and on the rodents were taken periodically examples of the results are summarized in TABLES 19 and 20 These preliminary observations suggest that the insecticide bait box method offers a cheap and efficient means of significantly reducing the plague vector flea populations affecting those native field rodents known to be implicated in sylvatic plague in the San Francisco region Nevertheless as stated above actual field control of wild rodent plague remains to be demonstrated

The control of fleas by contact insecticides having residual properties has correctly taken a place of primary importance in plague control On the other hand workers in this field must be prepared for the development of flea resistance to some of the insecticides Simmons (1954) has reviewed reports on such resistance and it is encouraging to note that no case of major resistance is known in important plague vectors in nature

In the long view sanitary measures construction of rat proof buildings public education and legal pressures must be intensified to reduce the popu

as an epidemic scourge but it remains entrenched as an animal infection external to man covering hundreds of square miles and perpetuating itself away from human habitation as a potential threat which may 'spill over to humans under circumstances neither wholly understood nor predictable The problem of plague control becomes more and more exclusively concerned with sylvatic plague control To speak of eradication would be utopian The so called eradication campaigns against ground squirrels in California and in certain parts of the Union of Soviet Socialist Republics during the early decades of this century and the continuous program of rodent poisoning in the Hawaiian Islands have not yet resulted in any significant decline either in the rodent populations or in the incidence of wild rodent plague

The ecologic picture of sylvatic plague developed in the foregoing sections of this paper suggests that control operations must be oriented to specific situations in which plague actually is transferred from wild to domestic sources or where a distinct threat of such transfer is obvious For this reason the chief concern must be in areas where wild rodent reservoirs are located near harborages containing rats such as villages suburban areas camps and garbage dumps in cities Beyond that control methods must be applied in specific situations where wild rodent epizootics in themselves threaten humans An outbreak in a national park or in the vicinity of an army encampment would deserve immediate intensive control It should be emphasized that control of *wild rodent plague is primarily an emergency measure with clearly limited* objectives Plague foci that threaten humans must be located and kept under surveillance so that control may be instituted rapidly when the need arises In certain cases prophylactic control of wild rodent plague foci may be justified

The possible methods of control are based on specific situations In cases where sylvatic foci impinge upon domestic rats a systematic program of rat flea and rat control is the easiest and most effective point of attack This can be accomplished by a routine program utilizing the anticoagulant poisons such as Warfarin and the newer insecticides including DDT chlordane and dieldrin DDT powder (5 or 10 per cent) still remains the residual insecticide of choice in rodent flea control Where small foci of plague occur near villages camps and other such areas the control of wild rodents with fast acting poisons and the control of their fleas are measures complementary to rat and rat flea control

Flea control in itself has become established as an important method of combating plague The use of DDT in urban areas and during outbreaks of rat borne plague has shown promising results (Kartman and Lonergan 1955a) Unfortunately there has been no adequate demonstration of plague control in wild rodent foci Field trials have shown that flea populations on wild rodents can be reduced by means of insecticidal dusting of rodent burrows (Davis 1951 Ryckman *et al* 1953) or by area dusting (Miles and Wilcomb 1953) but no actual demonstration of plague control by these methods is available

Whereas the insecticidal dusting of ground squirrel burrows is feasible because their entrances are easily located the control of fleas affecting the

small native field voles and meadow mice is difficult because their nests are hidden and do not occur in towns or community groupings as do those of the larger colonial rodents Furthermore wholesale dispersal of insecticidal dusts may not be safe in many areas near human habitation or human activity where the smaller rodents are found and where they present the chief potential for the spread of plague For these reasons flea control methods were developed in the plague endemic zone of Hawaii in which a DDT bait box was used (Kartman and Lonergan 1955a) The apparatus consisted of a covered bait station with DDT filled sacks hung at opposite entrances so that the rodent would receive a shower of DDT powder on entering and leaving the box The theory behind this method was that the rodent itself would become the vehicle by which the insecticide was dispersed to its nest and its mates In practice this method not only controlled those fleas on the rats but provided evidence suggesting that fleas in the nests also were affected Excavation of rat burrows showed a complete lack of fleas in the area of the DDT bait box whereas nests were 100 per cent infested in a check area and showed an average of about 25 fleas per nest Analysis of nesting material in one instance only showed DDT residues amounting to 0 72 mg In contrast to these results Wagle and Seal (1953) reported that DDT could be detected only in the first few inches of rat holes when the insecticide was applied by insufflation of the burrows Live fleas were found in 14 of 44 nests examined

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In the long view sanitary measures construction of rat proof buildings public education and legal pressures must be intensified to reduce the popu



FIGURE 6 End view of insecticide bait box in field operation. Note bait container DDT powder and rodent trail in foreground.

lations of domestic rats, especially those in the vicinity of wild rodent plague foci. If sylvatic plague can be isolated and returned to its former status of a purely wild disease rising and falling in a state of nature in its delimited foci the danger to man will have been reduced to the ultimate minimum. As the population of the country increases and our mechanical civilization encroaches

TABLE 19
EFFECT OF 5 PER CENT DDT POWDER IN BAIT BOXES ON THE INCIDENCE OF FLEAS ON *MICROTUS CALIFORNICUS* IN SAN FRANCISCO

Period	Treated plot (DDT treated bait boxes Aug 27 to Sept 27)				Check plot (150 feet from DDT treated plot)			
	Dates (1956-1957)	Number animals/Number fleas	Mean fleas	Per cent Microtus infested	Dates (1956-1957)	Number animals/Number fleas	Mean fleas	Per cent Microtus infested
Pretreatment	8/15-8/23	64/343	5 4	92 2	8/8-8/9	11/49	4 4	54 5
Treatment	9/5-9/26	162/2	0 01	1 2	9/11-9/13	27/200	7 4	78 3
Post treatment	10/2-10/17	206/63	0 3	21 4	10/9-10/17	37/176	5 5	99 9
Post treatment	10/23-11/27	239/274	1 1	50 6	10/23-11/27	109/564	5 2	88 9
Post treatment	1/8-2/7	56/59	1 0	41 1	1/8 2/7	40/126	3 2	82 5
Post treatment	3/19-5/2	63/58	0 9	42 8	3/19-5/2	34/46	1 3	52 9

TABLE 20
EFFECT OF 5 PER CENT DDT POWDER IN BAIT BOXES ON FLEA INCIDENCE IN NESTS OF *MICROTUS CALIFORNICUS* DURING THE POST TREATMENT PERIOD

Treated plot						Check plot					
DDT treated bait boxes Aug 27 to Sept 27						150 feet from DDT treated plot					
Dates (1956-1957)	Days after treatment	Number nests	Mean fleas per nest		Mean amt DDT (mg)	Dates (1956-1957)	Days after treatment	Number nests	Mean fleas per nest		Mean amt DDT (mg)
			Nests	Infested nests					Nests	Infested nests	
10-5	8	7	0 1	1 0	2 1	10-17	20	7	9 0	12 6	0
12 19	83	4	0 2	1 0	1 1	12-19	83	3	14 6	27 0	0
2-6	137	3	0	0	1 0	2-6	132	4	11 5	12 0	0
4-10	195	4	33 5	33 5	0	4-10	195	4	38 5	77 0	0

upon the suburban hinterland sylvatic plague will be increasingly subjected to the cleansing process that marks social progress

Summary and Conclusions

A general review of current problems and the present state of sylvatic plague in the western United States is given Historically emphasis has shifted from the large colonial rodents such as ground squirrels to the small inconspicuous native field voles and mice such as *Microtus* and *Peromyscus* The coexistence of these small rodents with domestic rats in the vicinity of human habitations and the prevalence of enzootic plague in these wild rodent populations poses a potential threat that has as yet not been fully elucidated Some of the present concepts included in the International Sanitary Regulations are fast becoming obsolete since they place major emphasis on classic urban and ship borne plague Cognizance must now be taken of the potential for transfer of wild rodent plague to domestic rats and thus to humans who could spread the infection abroad by modern rapid means of transportation

Brief accounts are presented of the rodents and fleas thought to be of importance in plague ecology in the San Francisco Bay region. Of several field rodents, the vole *Microtus californicus* is thought to be the chief plague reservoir because of its high degree of resistance to *Pasteurella pestis* and the fact that it was found to be parasitized by the important vector species as well as by more than 90 per cent of all fleas of all species taken in the area. The fleas *Hystrichopsylla linsdalei* and *Malaraeus telchinum* the two most prevalent species, were both found to have relatively high natural plague infection rates during epizootic incidents. Of the two *H. linsdalei* is an efficient vector by blocked individuals and *M. telchinum* transmits primarily by mass mechanical means. The former is probably the primary plague vector in this region whereas the latter may be a secondary vector of importance. The potential for transfer of wild rodent fleas to domestic rats was experimentally demonstrated with radioactively tagged *M. telchinum*, *M. californicus* and *Rattus norvegicus*. It was also shown that plague infection rates in individual fleas taken in the field could be determined and that this method compared favorably with the established method of plague detection by the inoculation of susceptible laboratory animals with flea pools.

Investigations on enzootic-epizootic plague in this region revealed that foci are quite delimited, that the disease is actually transferred from wild rodents to rats, that an increase in plague incidence in fleas and in rodents is not necessarily accompanied by a decimation of the wild rodent population, that an apparent epizootic may occur simultaneously with a rise in the *Microtus* population, and that sylvatic plague may remain dormant and difficult, if not impossible, to demonstrate for periods of eighteen months or more and then suddenly become resurgent.

Although relatively few cases of human plague have been traced directly to sylvatic sources, today's increasing suburbanization is of primary concern since it implies a period of "joint tenancy" between wild rodents, domestic rats and humans. An account of modern methods of diagnosis and treatment of plague is given and certain problems arising from a recent fatal human case are discussed.

Finally, sylvatic plague control is distinguished from urban control. The primary importance of the control of the domestic rat and the rat flea is emphasized. Sylvatic plague control is conceived of as an emergency measure or as a routine to be established in special instances as an adjunct to domestic rat and rat flea control. Recent field studies with an insecticide bait box method are summarized and the current limitations of sylvatic plague control are discussed.

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ANIMAL DISEASE AND BIOLOGICAL WARFARE

By Jack H Hempy

Office of the Surgeon General United States Air Force Washington D C

Any discussion of biological warfare (BW) and defense against it must include a serious consideration of animal diseases[1] The close and vital relationship of animal disease to human health and well being in the field of public health holds equally true in the field of BW

By way of definition BW is the intentional use of living microorganisms or their toxic products for the purpose of destroying or reducing the military effectiveness of man This includes damage to or destruction of food sources and supplies The military objective as in the case of all weapon systems is to reduce the will or capability to wage war

The statement has been made that BW is public health practice and procedure in reverse This is a common misconception The difference rests in the word deliberate BW is the deliberate use of natural disease agencies the inherent potential of which has been exploited by scientific research and development resulting in the production of BW weapon systems

Medical and military histories both provide us with the proper background for a review of modern day concepts of BW They tell us that military campaigns and troop concentrations have always provided fertile fields for naturally occurring epidemic disease and furthermore that infectious disease has often been the critical factor in the outcome of a military campaign Bubonic plague was credited with stopping the Crusaders at the very gates of Jerusalem Dysentery probably caused more casualties in Napoleon s Grand Army than did enemy firearms Typhoid fever and the dysenteries seriously hampered both sides in the Civil War and in the Spanish American War Even as late as World War I infectious disease was a controlling factor in some campaigns It is quite clear that typhus fever prevented the Germans from carrying through their Balkan campaign In World War II infectious diseases continued to hamper military forces materially Malaria ranked high as an enemy to United States forces in the Mediterranean and Southwest Pacific theaters Scrub typhus alone accounted for about 7000 casualties in the latter area Finally despite *our most modern sanitary and preventive medicine practices* we know that enteric infectious Japanese B encephalitis and hemorrhagic fever posed serious problems to the armed forces of the United States in Korea

What of the deliberate use of infectious disease for military purposes? History records many instances of it In the Fourth Century B C for example Alexander the Great attempted such exploitation by catapulting the bodies of dead men and animals over the walls of besieged cities It is recorded that smallpox was spread successfully among the American Indians during the French and Indian War by the distribution of blankets contaminated with purulent smallpox material In more modern times during World War I the Germans infected with glanders horses that were consigned from the United States to the Romanian cavalry In World War II German occupation forces

in Eastern Europe were said to have been the target of local sabotage efforts with bacteriological agents

Obviously these efforts were small local makeshift and unorganized It was not until early in World War II that an officially planned program was devoted to research and development in biological warfare This work has continued to the present time as a recognized activity of the United States Department of Defense Responsibility for carrying out this program was delegated to the Department of the Army which in turn assigned the operational responsibility to the Army Chemical Corps The major portion of the research and development is conducted at Fort Detrick Frederick Md Close liaison is maintained with other federal agencies in the United States having defensive responsibilities including the Federal Civil Defense Agency the Public Health Service the Food and Drug Administration and the Department of Agriculture The fact that a program of research and development continues in permanent facilities constructed for the purpose can be considered as recognition of the potential effectiveness of this weapon and thus of the defense problems that we must be prepared to meet

The nature of BW dictates its use primarily as a strategic weapon The major reason for this is that it has no quick kill effect The incubation period of infectious disease plus a variable period of illness even before a lethal effect render this weapon unsuitable for hand to hand encounter A man can be an effective fighting machine throughout the incubation period of most infectious diseases Thus it is logical to assume that an enemy would probably consider this weapon as primarily suited for attack on static population centers such as large cities Consequently it would appear that a logical approach in BW for an enemy would be to create an aerosol or cloud of the agent over the target areas

A number of unique medical problems will be created if and when man is exposed to an infectious agent through the respiratory route rather than by the natural portal of entry Botulinal toxin for example is several thousandfold more toxic by this route than when given *per os* In some instances a different clinical disease picture may result from this route of exposure making diagnosis difficult In tularemia produced by aerosol exposure for example one would not expect to find the classic ulcer of rabbit fever on a finger

What about agents? There are a number of them that an enemy might select from the several classes of microorganisms (bacteria viruses rickettsiae and fungi) There are however certain general characteristics that would apply in making a selection

An enemy obviously would choose an agent that is believed to be highly infectious Agents that are known to cause frequent infections among laboratory workers such as those causing Q fever tularemia brucellosis glanders and coccidioidomycosis all belong in this category

Any agent selected would probably possess sufficient viability and virulence stability to meet realistic minimal logistic requirements In this connection it should be capable of being disseminated without undergoing excessive destruction Moreover it should not be so fastidious in its growth requirements as to make production on a militarily significant scale improbable

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An aggressor would seek minimal naturally acquired or artificially induced immunity in a target population A solid immunity in the target population is the one effective circumstance whereby attack by a specific agent can be neutralized It must be remembered however that there are many agents for which there is no solid immunity and that a partial or low grade immunity may be broken by an appropriate dose of the agent

There is a broad spectrum of agents from which selection might be made for a specified military purpose An enemy might choose an acutely debilitating agent one that produces chronic disease or one that is highly lethal

It is possible that certain mutational forms such as drug resistant strains may be produced Mutants may be developed with changes in biochemical properties that are of importance in identification All these considerations are of critical importance in considering defensive measures However despite some of the unsubstantiated claims that have been made from time to time the likelihood of creating an entirely new agent of unique virulence or new disease producing capacity is extremely remote Even the remarkable genetic progress made in recent years in the production of bacterial transformation does not warrant deviation from this opinion at the present time

There are other general considerations in connection with BW agents that merit some consideration

Biological agents are of course highly host specific They do not destroy physical structures as is true of high explosives This may be of overriding importance in considering some military objectives

One must be unremitting in emphasizing that there is no secrecy concerning agents that might be included in an over all BW arsenal Only certain agents will meet the general and specific BW requirements and we and any potential enemy both know them The production of these agents is not like the invention or rather the synthesis of a new chemical poison One frequently hears it said that If we only knew on what agents our potential enemies were working we should know against what to defend ourselves This is the kind of statement that might be made by an ostrich before burying its head in the sand It would be more appropriate to ask ourselves What are we doing about it? Are we doing enough?

In defense against such attacks we must also consider that biological agents are suitable for delivery through enemy sabotage We could let our imaginations run wild in this regard I might mention a few obvious areas of significant importance Food processing including the preparation of soft drinks and the processing of milk and milk products constitutes an enormous industry that is subject to sabotage Huge industries are also involved in the production of biological products drugs and cosmetics that are vulnerable to this type of attack I mention these areas because sabotage in them would be far reaching in its consequences

The possibility of overt military delivery of biological agents from appropriate disseminating devices also must be considered It should be no more difficult to deliver such devices than other weapons The same means of delivery whether airplanes submarines or guided missiles should be usable

If it is possible for an enemy to drop an atomic bomb on a city it should be equally possible to put a cloud of some biological agent over that same city

Another defense consideration is the possible use of biological agents for the reduction or destruction of agricultural crops and domestic animals—in other words antifood biological warfare The importance of food particularly during war needs no emphasis Actually food production is of major concern to most countries even during peacetime

BW may find its greatest effectiveness when used against animals We must also consider that the epizootic potential of antianimal agents would be exploited by an enemy Antifood including antianimal BW could play a decisive role in any war not decided at its onset

Thus far we have touched on certain inherent capabilities of BW and the general requirements its agents should possess In so doing we find that BW might be employed overtly or covertly against man livestock and crops With this as a framework for defensive thinking let us now consider a few of the general features of the problem of defense

It is worth while to emphasize that there is a vast amount of medical knowledge in existence that can be useful We have had long medical and epidemiological experience with infectious diseases in both man and animal We have a vast public health effort in existence at the federal state and local levels Our preventive medicine practices and methods for disease control are at a high level of efficiency All of these are positive values that can be drawn upon now and in an emergency

This leaves no room for complacency however and we must avoid being lulled into thinking that these aids would render BW ineffective Unfortunately this is not the case All of these techniques have been developed over the years for dealing with naturally occurring infectious disease The military exploitation of massive amounts of highly infectious agents through unusual portals of entry creates many new problems for which these procedures were not designed and against which we have no experience For example public health practices have been developed for delivering wholesome food to all inhabitants in a community and today we take this for granted On the other hand there is no known public health procedure that will deliver sterile air to all inhabitants of a city Defense against a massive biological aerosol is a new and critically serious problem

In the light of such a possibility prophylaxis by active biological immunization may well be considered the most important defensive procedure of all A number of effective immunizing materials are already available for some infectious diseases On the other hand there are a number of potential BW agents against which there is no known method of immunization In other cases the value of the immunizing material continues to be questionable

Consequently we must encourage all possible research that is devoted to the development of new methods of active immunization or the improvement of existing ones Ancillary research dealing with host parasite relationships will also have positive defense value As a further incentive research of this nature has great peacetime value

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Part VII Selected Chronic Diseases

A COMPARISON OF RHEUMATOID-LIKE ARTHRITIS IN SWINE AND RHEUMATOID ARTHRITIS IN MAN

By Dennis Sikes

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The problem of arthritis in man, his domestic animals, and other members of the vertebrate subkingdom dates to antiquity. The earliest known case of multiple arthritis is in a fossil vertebrate of a large swimming reptile, the plate carpus, which is calculated to have lived approximately 100 000 000 years ago, a skeleton of which is in the Museum of Natural History at the University of Kansas,[1] Lawrence, Kan. According to Hollander[1] chronic arthritis of the spine was present in the ape man of 2 000 000 years ago, as well as in the Java and Lansing men of 500 000 years ago, and in Egyptian mummies dating to 8000 B.C. The Romans built baths throughout their empire because of this disease.

Hollander[1] estimates that more than 10 000 000 people in the United States have some form of rheumatic disease, and that more than 4 500 000 of these have some form of arthritis. Of those with arthritis, 190 000 are completely disabled and another 1 143 000 are partially disabled.

Swine erysipelas is an infectious and contagious disease of swine that causes a relatively high morbidity with a low mortality rate in most affected herds. The causative agent *Erysipelothrix rhusiopathiae* derives its name from the Greek *erysipelas* = red skin, *thrix* = a hair or thread, *rhusis* = reddish, and *pathos* = a disease. According to Bergey[2] the swine erysipelas organism has been assigned to the family Corynebacteriaceae and genus *Erysipelothrix*. The organism was described by Pasteur and Dumas,[3] and Loeffler[4] is credited with the first observation of the organism in swine. He observed the organism in the blood vessels of a pig that apparently had died from swine erysipelas and thought that it was similar to Koch's *Erysipelothrix muriseptica*. Later Rosenbach[5] reported his classic studies of the organism and suggested the names *E. muriseptica*, *E. porci*, and *E. erysipeloides* for the mouse, pig, and human organisms, respectively.

Konst[6] indicates the possibility that two variants of *E. rhusiopathiae* exist, the highly virulent one prevalent in Europe and a less virulent one in North America. The observations of Breed[7] do not support this concept, for the disease is spreading in North America and is of great economic importance. It may be that the more virulent strains have been imported in swine originating in Europe. The relationships of the microorganisms isolated from man, sheep, chickens, turkeys, and swine have been studied by Beaudette and Hudson,[8] Klauder and Harkens,[9] and Van Roekel, Bullis, and Clarke,[10] who were unable to show any significant cultural differences between strains.

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Another defense procedure of potential value lies in the possible prophylactic use of antibiotics and chemotherapeutic drugs However here again we must avoid being lulled into a false sense of security For example published articles have expounded the thesis that our enormous antibiotic industry has made BW obsolete This idea verges on the ridiculous for several reasons To permit such reasoning to guide our defense planning would be dangerous indeed There are many potential BW agents for which there is no known effective antibiotic or drug Among these may be mentioned *Coccidioides immitis Histoplasma capsulatum* and more importantly from a BW standpoint most of the filtrable viruses Moreover *what of the not unlikely possibility of drug resistant agents?*

Much more space than is available here could be devoted to still other and equally significant defensive problems including detection, disease reporting decontamination and protective equipment As stated above my purpose here is merely to suggest some areas of investigation that are pertinent to the problems of defense against BW Unfortunately in the world as it is today we must give serious attention to the negative aspect of the ever growing body of evidence that man s health and well being progress in direct relationship to his successes in the prevention control and treatment of animal diseases

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FIGURE 1 Extensive erythema of the skin of a white hog with acute swine erysipelas following intravenous exposure to *Erysipelothrix rhusiopathiae*

glomerulosa was infiltrated with many leukocytes and complete or almost complete disappearance of this zone occurred in some cases Numerous foci of round cells were often scattered throughout the fascicular and reticular zones The medulla usually appeared normal but some showed atrophic or degenerative changes In advanced cases of chronic arthritis a disruption of the normal architecture of the glomerulosa and a reduction in thickness occurred

Collins and Goldie[18] and Sikes Neher and Doyle[19] observed in the acute stages of *E rhusiopathiae* arthritis that the principal changes in the joints were characterized by vascular engorgement of the joint capsule and synovial tissues and more or less edema The effusions were turbid or serosanguineous or mucinous The synovial villi showed beginning proliferation extensive vasodilation engorgement and incipient lymphocytic infiltration

In arthritis of two months duration greater proliferation and less edema were observed Much proliferation of the cells covering the hypertrophied synovial villi was present The villi contained young highly vascular connective tissue infiltrated with plasma cells and lymphocytes A striking feature was the dense collection of lymphocytes (FIGURE 3) The villi of arthritic joints of six months or longer duration frequently resembled granulomatous polyps (FIGURE 4) Very few polymorphonuclear leukocytes were observed and the infiltrating cells were principally lymphocytes plasma cells and eosinophils Less exten

Natural outbreaks of *E rhusiopathiae* infections have been recorded in sheep cattle horses turkeys ducks peacocks pheasants and farm raised mink (Beaudette and Hudson[8] Graham Levine and Hester[11] Marsh[1] Paterson and Heatley[13]) Also according to Klauder Righter and Harkens[14] human infections have occurred from handling fish

For more than a century in Europe the economic importance of swine erysipelas has been emphasized, but it has reached serious proportions only in the last two decades in the United States According to a study made by the Organization for European Economic Cooperation (OEEC)[15] all of the European nations reported that the disease was affecting many herds of swine

In the United States arthritis in swine has rated second or third in responsibility for the number of swine carcasses condemned at federally inspected meat packing plants for the last ten years according to the annual reports of the Chief of the Bureau of Animal Industry United States Department of Agriculture[16] The number of carcasses condemned in 1952 was 22 196 This represents only a small fraction of the true incidence of the disease complex because the animals sent to slaughter for human consumption are thought to be healthy It is generally known that lame animals are condemned and swine producers do not ordinarily send arthritic animals to market Obviously, the great majority of affected animals remain at the farm until they recover or die

Pathological Changes in Acute and Chronic Swine Erysipelas

The lesions of swine erysipelas are varied Sikes *et al*[17] have shown that in animals that die of the acute septicemic form of the disease pathological changes observed at necropsy may be no different from those observed in any other septicemic disease The skin lesions perhaps are more nearly pathognomonic than any other finding (FIGURE 1) Damage to the blood vessels of the skin resulting in the peculiar type of erythema presumably was due to the action of the organism and its by products The histopathological changes occurred principally in the papillae and in the subpapillary and upper layers of the dermis The blood vessels were distended and the papillae were enlarged and showed marked hyperemia Frequently small hemorrhages were observed in the connective tissue elements of the papillae (FIGURE 2) Numerous foci of round cells and necrotic areas were present In the urticarial lesions marked edema of the skin occurred in the wheals Dilation of the vessels and numerous thrombi were present Capillary hemorrhages appeared especially in the papillary layer The chief difference in the skin lesions of swine with the acute septicemic form and those showing urticaria was in the degree of damage done

They also observed pronounced white blood cell dyscrasia A significant and marked leukopenia lymphopenia neutropenia and a monocytosis occurred in the acute phase of the disease This appears to be the most critical phase of the disease as related to survival or death of the animal In acute swine erysipelas the adrenal glands were enlarged and frequently subcapsular hemorrhages were observed In chronic cases lasting two months or more the adrenal glands were often swollen to twice the normal size and were very soft the zona



FIGURE 3 Proliferation of the synovial villi with accumulati n of roun l cells ×1/0

changes first then lesions of muscles nerves tendons bursae integument and serous membranes of the heart kidneys reticuloendothelial structures and intestines Collins [9] also places great importance on the joint changes supplemented by other tissue change Both of these authors agree that the microscopic pathology of synovial membranes in rheumatoid arthritis is quite characteristic and that the basic pattern may be considered pathognomonic of rheumatoid arthritis as it is not exactly duplicated in any other articular disease The basic pattern consists of hypertrophy vascularization cellular infiltration accumulations of inflammatory cells in follicle like collections and pannus formation

Several components of the synovial membrane undergo hypertrophy The endothelial lining may reach several cells in thickness The stroma is increased in amount being fibrillar infiltrated with fat and obscured by cellular filtrate in some cases The villous folds of the lining become hypertrophied causing the villi to be enlarged

Vascularity of the synovium may be profuse or modest The walls may be thickened and whorls of fibrous tissue surround the medial coats The veins frequently are dilated

The cellularity is variable from case to case Lymphocytes plasma cells reticuloendothelial cells polymorphonuclear leukocytes fibroblasts and a few giant cells appear in the subendothelial layer



FIGURE 2. Hemorrhages in the skin of an experimentally produced case of acute swine erysipelas. ×200.

sive destruction of cartilage and bone than that which characterizes suppurative arthritis in other species was observed. The synovial effusions were nonpurulent and contained mostly monocytes, lymphocytes, and a few synovial cells.

In advanced chronic arthritis with pannus formation of subchondral origin, marked fibrosis of the adjacent marrow spaces, increased vascularity, and collections of lymphocytes occurred. In those cases in which the pannus apparently originated from the synovial membrane, subchondral fibrosis was less likely to occur (FIGURE 5). Pannus attachment to the articular cartilage was accompanied by erosions and destruction of the cartilage. In advanced cases with little or no synovial fluid, intra-articular fibrous adhesions formed apparently in an attempt to immobilize the joint.

Radiograms revealed osteophyte formation with apparent ankylosis of the carpal and tarsal joints (FIGURE 6). Also, the articular processes of the vertebrae frequently appeared to be fused. Irregular ossification and rarefaction of bones adjacent to the joints were frequently observed. In hogs that spontaneously recovered, no gross abnormalities were observed.

Pathological Changes in Rheumatoid Arthritis in Man

The pathological alterations of the tissues in rheumatoid arthritis are widespread throughout the body, according to Hollander,[1] who emphasizes joint



Figure 6 Photograph of an X ray showing closure of carpal spaces

Follicle like collections of chronic inflammatory cells are present in the synovial membranes of rheumatoid arthritis These follicles consist mainly of lymphocytes and some reticuloendothelial cells In some cases they are sparse in others they are collected densely throughout subendothelial tissues

The formation of pannus is a most important phenomenon in the pathological process It may arise from the lateral margins of the synovium or occasionally subchondrally In any case it may appear reddened and rough with irregular margins resulting in the destruction of cartilage In these cases fibrous tissue covers the epiphyseal bone thus leading to fibrous intra articular adhesions

The pathological changes in epiphyseal bone near the affected joint are osteoporosis follicle like accumulations of lymphocytes in marrow spaces and granulations absorption of bone eburnation of subchondral bone and production of osteoid tissue in the marginal zone

In cases of spondylitis the discs show progressive atrophy with narrowing of intervertebral spaces Osteoid tissue grows through the degenerated discs and later forms bone resulting in bony adherence of the vertebrae In late cases intervertebral ligaments may become calcified other bones such as ischium and pubis may be affected

Discussion

Erysipelothrix rhusiopathiae infection in swine may be acute subacute or chronic Frequently arthritis is a sequel to these forms of infection (Sikes Neher and Doyle)[17] A distinctive feature of the pathological process is its chronic proliferative nature without the formation of pus and destruction of

FIGURE 4. Granulomatous polyp formation in advanced arthritis. ×170.



FIGURE 5. Section of carpal bone showing lateral pannus formation extending between articular surfaces.

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bone that is characteristic of other bacterial infections of joints Few or no polymorphonuclear leukocytes are present The synovial effusions are serosanguineous and mucinous similar to those observed in rheumatoid arthritis in man

The formation of hypertrophied synovial fringes whose villi become bulbous with focal collections of lymphocytes resembling germinal centers is common to both diseases The villi become greatly enlarged and granulomatous later in the disease and are very active even in the absence of an infectious agent Sikes Neher and Doyle[1] were not able to isolate an infectious agent after 226 days after exposure yet the disease persisted in an active form with periods of remission and exacerbation Collins and Goldie[18] believe that one of three things may happen in chronic arthritis due to *E rhusiopathiae* infections (1) the lesions may resolve and the joint may recover (2) healing of the damaged tissue may take place by fibrosis and this may include the formation of intra articular adhesions, or (3) the synovial granulation tissue may assume certain properties of a benign neoplasm and persist and extend in the absence of the exciting microorganisms thus maintaining the arthritis in a state of clinical activity Just what factors are responsible for this excitement of the arthritic condition in the absence of an infectious agent remains to be discovered Sikes, Neher and Doyle[19] have suggested that perhaps adrenal and kidney pathology play an important role in this syndrome

Pannus formation destruction of cartilage at the site of pannus attachment and subchondral cellular reaction are common to *E rhusiopathiae* arthritis of swine and rheumatoid arthritis of man Intra articular fibrous adhesions and rarefaction of the bone as revealed by X ray are also common to both diseases Gross destruction of bone does not occur in either disease Perhaps no further analogy should be drawn between the two maladies until the cause of rheuma toid arthritis in man is known

Summary

A description of the pathology of *Erysipelothrix rhusiopathiae* infections in swine is given the major pathological joint changes in rheumatoid arthritis in man are discussed and a comparison of the pathology of *E rhusiopathiae* arthritis in swine and rheumatoid arthritis in man is made

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workers on a variety of experimental animal neoplasms there has emerged a tentative explanation for some at least of the apparent contradictions between the observed epidemiology of neoplasm in natural populations and the concept of viral transmission A group of transmissible neoplastic diseases of sheep has been chosen to serve as a possible model for the study of neoplasms occurring in natural populations

These sheep diseases occur in a variety of forms ranging from infiltrative pneumonia to metastasizing carcinoma In this paper they are considered to constitute together a complex of related diseases for convenience they are referred to here as sheep pulmonary adenomatosis or SPA even though a few forms do not show adenomatous features The reason for considering these various diseases as members of a single group will be discussed below

Considered from either viewpoint the SPA complex is of particular interest since (1) at least some and probably all forms are infectious (2) members of the SPA complex range in pathology from inflammatory or infiltrative to carcinomatous and (3) various forms of SPA while almost certainly of viral etiology can occur in both epizootic and enzootic form thus resembling both the picture of classic viral transmission (epizootic) and that of natural tumor epidemiology (occurring at low rates or enzootic) in which viruses are not ordinarily thought to be implicated Examples of the last case are certain tumor conditions such as mouse leukemia [7] fowl lymphomatosis [8] and the mouse mammary gland carcinoma [9] which occur at enzootic levels in wild populations and yet were shown after the development of inbred strains of the host species involved to be propagable by means of viruses just as are many of the traditional epizootic inflammatory diseases The study of SPA therefore may be useful in formulating unifying theories concerning the pathology and the etiology of inflammatory and neoplastic diseases and also in understanding the etiology of natural tumors

The History of SPA

SPA was first recognized by J M Fadyean in 1888 as a rather rare adenomatous condition occurring in certain flocks of sheep in England [10] A report from Germany followed in 1899 [11] A commercially significant infiltrative pneumonia was observed in South Africa in 1904 by W Robertson[12] and reported there again in 1915 by D Mitchell [13] A chronic progressive pneumonia with some adenomatous overtones was reported from North America by H Marsh[14] in 1922 and a more adenomatous form was reported from France by M Lesbouyries and A Bonnac in 1940 [15] Perhaps the best studied forms of SPA from the viewpoint of epidemiology and mode of transmission are two diseases of Icelandic sheep One pulmonary adenomatosis is largely adenomatous in character the other *Maedi* (an Icelandic word meaning dyspnea) is of the infiltrative type Both forms which occur as separate but presumably related diseases were introduced by two karakul rams in a single shipment from Halle Germany in 1933 The diseases were not widely disseminated in Germany at that time and did not appear in Iceland until 1936 nevertheless it was possible to trace the infections back to the original carriers that had arrived three years earlier [16] The two diseases subsequently became grave commercial problems

THE PULMONARY ADENOMATOSIS COMPLEX IN SHEEP*

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Introduction

With the discovery that certain tumors can be transmitted by means of cell free extracts interest naturally arose in possible comparisons between neoplastic viruses on the one hand and viruses of the traditional sort on the other As a consequence of this interest attention has been drawn to situations in which viruses of classic inflammatory disease can give rise to disease that is histologically neoplastic For example interest has revived in A Borrel s studies of sheep pox and of fowl pox [1] This investigator showed that the viruses of these diseases induced both necrotic and proliferative lesions and were in fact once designated as contagious epithelioma [1] E Roux observed pulmonary adenomatosis in sheep infected with sheep pox virus [2] and papillomas have been observed at the sites of the lesions of contagious pustular dermatitis in sheep [3] Smallpox cowpox rabbit pox mouse pox and other members of the pox group of viruses also can show a proliferative phase as well as the better known necrotizing phase

Conversely viruses that ordinarily cause tumor formation can produce inflammatory and necrotizing disease under certain conditions according to dosage inoculation route host age and host resistance F Duran Reynals has demonstrated the inflammatory character of the Shope fibroma virus in newborn rabbits [4] and of the Rous sarcoma virus in young chickens [5] In some recent work Duran Reynals[6] has shown that mice pretreated with methylcholanthrene and cortisone and then inoculated intradermally with vaccinia virus develop tumors rapidly in the sites of the healed vaccinia lesions Although at present it is not possible to say whether vaccinia participates directly in the formation of those neoplasms it can be said at least that the vaccinia lesion appears to be an optimal ground for the development of malignancy

A second important question for students of neoplasia is the role of viruses in the production of tumors in general From observations made by many

* The work reported in this paper was supported in part by the American Cancer Society Inc New York N Y

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another form of SPA (FIGURE 1b) that has been named epizootic pulmonary adenomatosis or Icelandic PA. Unlike *Maedi* which seems to have a somewhat longer development period, Icelandic PA shows considerable proliferative change with slight to-pronounced infiltration,[19] much of which does not appear to be of an irritative nature. Metastasis has not been observed. Spread of the disease is thought to have been facilitated by the Icelandic practice of "huddling" or closely confining the sheep in barns during the winter. This disease together with *Maedi* had rendered commercial sheep raising all but impossible until both diseases were eradicated within a few years by a radical slaughter program following their recognition as contagious diseases. At this writing neither disease has been observed in Iceland for more than 4 years although it was not uncommon in the mid 1940s for 60 per cent of infected flocks to be lost within the span of a few years.[16]

A situation similar to that in Iceland exists in South Africa where a form of SPA very similar to *Maedi*, Graaff Reinet disease, occurs side by side with *jaagsiekte*, a form more similar to or identical with Icelandic PA.[20, 21] Graaff Reinet disease (FIGURE 2a) seems to involve infiltration of rather more uniform character than in the case of *Maedi*, there being less tendency toward the formation of clusters of lymphocytic cells and a somewhat shorter development period. Histologically, Graaff Reinet disease, so named in recognition of the high incidence of that form near the Graaff Reinet experimental station in the Union of South Africa, resembles progressive pneumonia and also lacks obvious features of irritation. On the other hand, *jaagsiekte* (FIGURE 2b) or

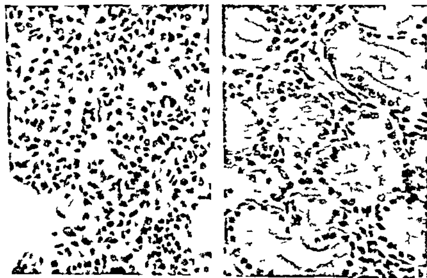


FIGURE 2. (a) Graaff Reinet disease, South Africa. Infiltrative with little sign of endothelial proliferation. ×200. (b) *Jaagsiekte*, South Africa. Adenomatosis with or without marked infiltrative change. ×200. (Specimens furnished by G. deKock, Liesbeck Clinic, Cape Town, and G. leRoux, University of Pretoria, Onderstepoort, Union of South Africa.)

in Iceland. Finally, a metastasizing adenocarcinoma somewhat resembling *jaagsiekte* (see below) in section was reported from Peru in 1945 by A. Cuba Caparo.[17]

The Pathology of SPA

Beginning at the "inflammatory" end of the SPA spectrum is an Icelandic disease, *Maedi* (FIGURE 1a). A highly contagious illness, *Maedi* requires about three years from the time of infection to manifest clinical signs; death usually follows a few months later. B. Sigurdsson[18] and Sigurdsson *et al.*[19] have shown, however, that development of the disease actually begins within a few months of exposure in the form of *numerous infiltrative nodules that progressively increase in number and size* until respiration is impaired sufficiently for the appearance of external signs. These consist of emaciation, rapid and shallow breathing, moist rales on auscultation, and general weakness. The signs invariably progress until death results. On necropsy, the lungs are massively infiltrated, presenting large grayish areas that seem to consist of small confluent nodules. The cut surface is firm or friable, often dry, while the weight of the lungs is threefold to fourfold that of normal lungs. The tissue is often sterile. On section (FIGURE 1a) the lungs are seen to be engorged with cells of lymphocytic type, with a tendency toward the formation of clusters. There is no sign of adenoma or of irritation. Necrosis is not prominent. A series of carefully performed experiments has shown clearly that *Maedi* is highly contagious by means of air and fecal contamination of drinking water.[18, 19] Also in Iceland, in territory largely different from that of *Maedi* but overlapping it, there occurs

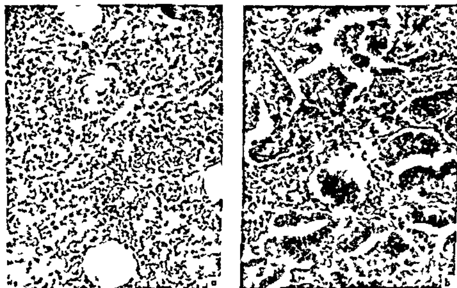


FIGURE 1. (a) *Maedi* of Iceland. Infiltrative, necrosis lacking. ×200. (*b*) Contagious pulmonary adenomatosis of Iceland (Icelandic SPA). Infiltration plus adenoma. ×200. (Specimens furnished by Björn Sigurdsson, University of Iceland, Reykjavik, Iceland.)

monia showing a marked tendency toward the early formation of peribronchiolar clusters of cells resembling lymphocytes As the disease progresses invasion becomes more generalized resulting in greatly thickened interalveolar walls and cuboidal transition of the alveolar lining In the later stages of the disease a striking adenomatous change often occurs as an accompaniment of the characteristic pneumonia (FIGURE 3b) Marsh[23] believes that Montana SPA like *Maedi* is transmissible on contact and although this author does not consider the adenomatous condition to be neoplastic it has not been possible in our experience to distinguish Marsh s material from lesions that have long been classed as neoplasms in human pathology Furthermore Montana SPA whether the pneumonia or adenoma form prevails is invariably progressive as in Iceland and in South Africa which does not seem to suit the hypothesis that the changes are hyperplastic reactions to nonspecific stimulation of normal lung tissue Although it is well known that in man at least many irritants such as poisons can cause pulmonary hyperplasia that somewhat resembles adenoma[24 30] hyperplasia does not in our opinion closely resemble legitimate cases of SPA The crux of the nomenclature problem seems to depend in the case of pulmonary adenoma upon whether metastasis is observed The fact that neither Icelandic South African nor Montana SPA has been observed to form metastasis is of somewhat diminished consequence however since closely related forms of SPA from France and from Peru do show metastasis

The French form of SPA *bouhite* derives its name from a patois term meaning panting Histologically it is close to Montana SPA with the reservation that adenoma generally accompanies infiltration and is often in fact the dominant feature of the disease Metastasis to the mediastinal lymph nodes or to subcutaneous lumbar lymphatic tissue has been shown in two cases[31 F] The infiltrative a pect of *bouhite* closely resembles lymphomatosis and the term malignant pulmonary lymphomatosis has been suggested by Lucam for this disease[32] and for others of the complex that show parenchymal infiltration by cells of the lymphocytic series in the absence of massive necrosis or obvious irritation Actually all forms of the complex show this leukotic aspect to some degree Predisposition toward *bouhite* may be associated with the ingestion of resinous matter in pine forests judging from the distribution of the disease in France although there is no positive evidence to support this theory[34] One case in fact has been reported from the Department of Seine et Marne where contact with pine resin is less likely[35]

As it appears in Peru (FIGURE 4) SPA cannot escape classification as carcinoma since lymph node metastasis has been observed[7] (FIGURE 4b) Infiltrative aspects are subdued The disease is recognized in the field by respiratory distress following exercise and by discharge from the nostrils of large amounts (often a liter or more a day) of mucous exudate presumably produced by the adenoma or adenocarcinoma[17] Except for the sparseness of the inflammatory change Peruvian SPA closely resembles *jaagsiekte* and other adenomatous forms of SPA Like them Peruvian SPA is invariably progressive The disease occurs preferentially at altitudes of several thousand feet

driving sickness, so named following the observation that the first clinical signs of the disease commonly appear during exercise, seems to be variable in the comparative amounts of adenomatous versus infiltrative changes that occur. *Jaagsiekte*, the prototype of SPA, is referred to here as representing the more adenomatous form of the disease. Both forms, however, occur in the same territory, and it does not seem possible to separate them into distinct entities since there is considerable gradation of one form into the other. Adenomatous cases, however, can often be distinguished clinically from infiltrative cases because the former tend toward the production of more or less large amounts of mucous exudate which is discharged through the nostrils. Metastasis has not been observed in either form.

The North American form of SPA, called "progressive pneumonia" or "chronic catarrhal pneumonia," is confined to Montana and a few other western states. The disease appears in range sheep and is commonly first observed in the spring of the year, infection possibly occurring during the winter. The subsequent development of the disease is typical of that of the SPA complex, afflicted sheep ("lungers") dying of asphyxiation or secondary bacterial pneumonia 3 to 12 months after the first appearance of clinical signs.[14]

As in Iceland and in South Africa, SPA of Montana presents both the infiltrative and adenomatous aspects of this disease, but both forms are more likely to occur in the same animal, the adenomatous form being less conspicuous. After a study of both forms, E. Cowdry and H. Marsh[illegible] concluded that *jaagsiekte* and Montana SPA were probably essentially the same disease. Histologically, the inflammatory aspect (FIGURE 3a) is an infiltrating pneu-

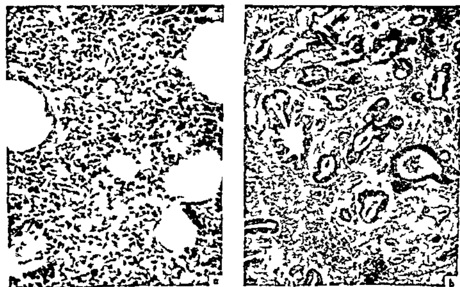


FIGURE 3. (a) Chronic catarrhal pneumonia, United States. Infiltrative form. ×200. (b) Chronic catarrhal pneumonia, United States. Adenomatous form accompanying the infiltrative form. (Specimens furnished by Hadleigh Marsh, Livestock Sanitary Board, Helena, Mont.) ×100.

by specimens taken from heavily diseased hosts[45] A virus came to be suspected as the etiological agent when SPA was observed to occur in epizootics in South Africa and Iceland At the present time there remains no doubt that at least some forms are virus induced Transmission by extracts of diseased lung has been effected with the Montana[43] Icelandic[15 43 44] and Central African[41] forms of SPA The Icelandic form *Maedi* apparently can be transmitted by air and by means of fecal contamination of drinking water[16] and Shirlaw[41] claims to have recovered a specifically antigenic virus after two serial passages of extracts of lung from cases of Laikipia lung disease (of Central Africa) in the yolk sacs of hen s eggs Many attempts to transmit the disease experimentally however have been complicated by the fact that adequate isolation of control animals is extremely difficult The difficulty of accomplishing effective isolation implies that contagiousness may be of a high order in spite of the fact that the attack rate is usually well below 30 per cent Another factor making for uncertainty in the results has been the length of the latent period which has been shown in some forms to be from 1 to 3 years in duration Some of the negative experiments were undoubtedly terminated before the development of clinical disease A few attempts have been made to transmit the disease by means of filtrates but it is felt that the results are not entirely conclusive because of the small numbers of animals used in the experiments or because of difficulties in maintaining strict isolation of inoculated sheep

In one experiment Dungal observed SPA in 3 of 8 Icelandic sheep receiving a Gradacol filtrate (0 9 mμ pore diameter) of glycerine solution through which sick sheep had been required to breathe In another experiment 6 of 38 sheep developed SPA 4 to 9 months after receiving injections of Berkefeld Chamberland or Seitz filtrates of infected lung In both experiments the uninoculated control animals remained free of disease[45] In an experiment performed by C Creech and W Gochenour[43] 23 sick sheep were shipped from Montana to Maryland where SPA is unknown and 96 Maryland sheep were exposed to the disease either by inoculation (81 sheep) or by contact (15 sheep) None of the sheep exposed by contact developed the disease although 4 of the 81 sheep inoculated with sterile extracts of diseased lung developed SPA in 3 to 12 months and 4 other cases of slight pneumonic lesions were noted on histological section at autopsy

The Serology of SPA

The attempt to establish several geographically separated and histologically varying diseases as members of a single complex certainly should include serologic studies of the agents involved and the distribution of antibodies in infected and uninfected flocks Unfortunately the agents involved have been and remain extremely elusive with the exception of the previously mentioned case reported by Shirlaw[4] The partially purified agent obtained by inoculating lung extract from sheep ill with Laikipia lung disease into the yolk sacs of embryonated hen s eggs fixed complement with high dilutions of serum from sick sheep but not with that from healthy sheep of the same area Formalin treatment of the preparation is reported to result in a vaccine that reduces the

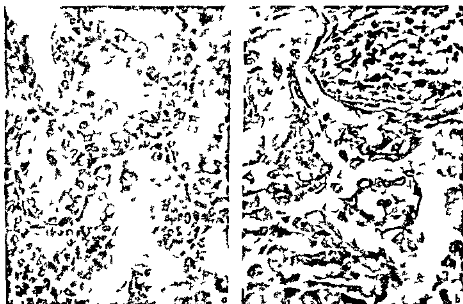


FIGURE 4. (a) Pulmonary adenomatosis of Peru. Adenomatous infiltration slight to moderate. ×250. (b) Lymph node metastasis, pulmonary adenomatosis of Peru. The same sheep as in (a). ×250.

The Epidemiology of SPA

Beginning in the 1920s SPA seemed to increase sharply in world-wide incidence and distribution, although part of the apparent increase was undoubtedly due to its recognition as an important disease in commercial flocks. SPA has also been reported, at one time or another, from The Netherlands,[38] Scotland,[37,39] Israel[40] and Central Africa.[41] In recent years SPA may have begun a natural decline in incidence, at least in Peru,[42] the United States,[3] and Iceland (even before the artificial eradication of the disease there),[43] possibly showing the declination of a long-term epidemic curve. In addition, many parts of the world have never reported the disease. The principal factor in distribution does not seem to be entirely one of host susceptibility, since sheep from regions free from SPA have been shown to be susceptible when inoculated with extract of diseased lung[44] or when moved to infected areas.[45] Certain breeds, however, such as the Adalbol, were discovered to be partially refractory to the Icelandic disease,[44] and others, including the Merino, to the South African disease.[46]

The contagiousness of SPA was suspected by its first describer, M'Fadyean, who believed it to be a form of verminous pneumonia caused by *Strongylus* infestation. Other workers, however, showed that worm infestation did not necessarily accompany the disease, and studies in Iceland failed to show a correlation between occurrence of SPA and infection with any particular parasite or microorganism.[44,47] At one time the sheep ked *Melophagus ovinus* was suspected as a vector in Iceland, but *Maedi* was not induced in sheep bitten

ment were allowed to mingle with the inoculated group and a few with those receiving only methylcholanthrene Sheep from these groups were sacrificed and examined at intervals

Eight sheep sacrificed at 4 months showed no significant lung lesions In 3 sheep sacrificed at 8 months however apparent incipient proliferative lesions of the pulmonary alveolar epithelium were found in both of the sheep that received infected lung extract (FIGURE 5 a and b) Here the alveolar sacs are

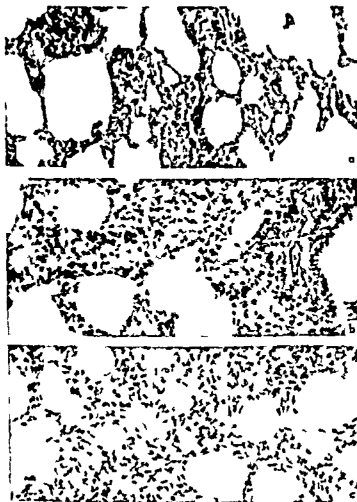


FIGURE 5 (a) Lung of an apparently healthy sheep sacrificed 8 months after the intravenous injection of extract from lungs infected with chronic catarrhal pneumonia (see FIGURE 3a) ×250 (b) Lung of similar sheep that received the same injection also sacrificed 8 months later ×250 (c) Lung of apparently healthy sheep sacrificed 11 months after the intravenous injection of the same extract ×250

natural incidence of disease from 30 per cent to about 1 per cent when the vaccine is administered within the first month of life Further evidence for the antigenicity of SPA agents is the observation made in Peru by one of us (A Cuba Caparo) that formalin treated suspensions of diseased lung emulsion are effective as vaccines No complement fixation tests have been done with the Peruvian form In a careful search no complement fixing activity was found in the Icelandic form *Maedi* All attempts to cultivate the agents of *Maedi* and of Icelandic SPA have failed and extracts of diseased lung prepared in various ways have been found to be devoid of the ability to fix complement with serum from sick sheep or to produce specific hemagglutinins [16]

Unfortunately, no general method for the demonstration of specific antigen in the lungs of diseased animals is available consequently, it has been impossible to determine whether serologic relationships exist between geographically separated forms of SPA

The Host Range of SPA

All evidence available indicates that the host range of SPA is extremely narrow the disease being transmissible only from sheep to sheep although pulmonary adenomatosis or infiltrative pneumonia have been observed in man [49,55] cats [56] guinea pigs [57] mice [53] chinchillas (E J) goats [3] horses [59] hogs [24] cattle [5] a tiger [24] a deer [60] and many other animals Our attempts to transmit Montana SPA to Swiss mice hamsters and chinchillas as well as to induce replication of an agent in embryonated hen s eggs have failed up to the present time Early speculation that pneumonia occurring in sheepherders may be derived from SPA infected sheep is now believed to be unfounded [1] PA has not been reported in man in Iceland although that country has suffered the most devastating attack rate of this disease in sheep [19] Over a period of a few weeks glycerol stored material from a case of PA in man did not give rise to lesions when inoculated into a monkey into rabbits and into guinea pigs [51] The possibility exists that cultivation of an agent of SPA in embryonated eggs or in tissue cultures might allow the demonstration of a wider host range than has been shown to exist

Experimental

In our experiments attempts have been made to transmit the North American form sheep progressive pneumonia to other sheep using lung tissue from diseased sheep The lung tissue was taken from diseased Montana sheep that were sacrificed for the purpose frozen on dry ice and shipped to our laboratory by Hadleigh Marsh of the Livestock Sanitary Board Helena Mont Sterile saline extracts were inoculated intravenously in lambs 1 to 7 days old some of which were simultaneously treated with methylcholanthrene by intraperitoneal injection in sesame oil to determine whether a carcinogenic agent might accelerate the development of the disease or shift its expression from infiltrative and inflammatory to a more neoplastic type Other groups received methylcholanthrene with no extract of infected lung and were separated from the inoculated lambs by means of a board fence A few lambs receiving no treat

ment were allowed to mingle with the inoculated group and a few with those receiving only methylcholanthrene Sheep from these groups were sacrificed and examined at intervals

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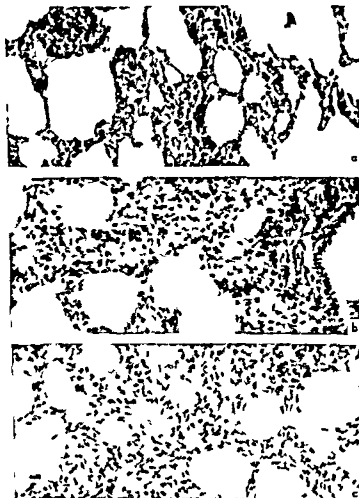


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lined in places by cuboidal epithelium Of those animals receiving lung extract one showed the infiltrative condition characteristic of Montana progressive pneumonia (FIGURE 5a) The nature of the lesions was the same regardless of whether methylcholanthrene was administered The 3 sheep that did not receive extract of infected lung showed no such proliferation

It was suspected at first that this early proliferation might develop into adenoma in sheep receiving both lung extract and methylcholanthrene, later sacrifices however, did not bear out that assumption Five sheep were sacrificed approximately 11 months after inoculation Of the 2 that received extract of infected lung, the one that did not receive methylcholanthrene showed slight but probably significant proliferation of alveolar and bronchiolar epithelium (FIGURE 5c) In 8 sheep (4 controls and 4 receiving lung extract) sacrificed 13 months after inoculation, the inoculated group showed nodes and consolidation in gross aspect and circumscribed nodules of lymphocytic infil

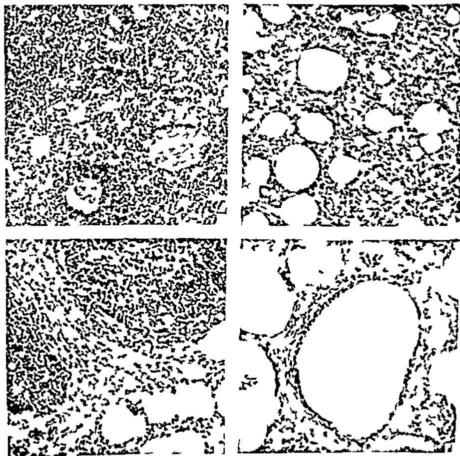


FIGURE 6 Lungs of 4 apparently healthy sheep sacrificed 13 months after the intravenous injection of the same extract used to induce the lesions shown in FIGURE 5 ×250

tration on section (these 4 cases are pictured in FIGURE 6) The picture represented is that typically seen in pulmonary adenomatosis of Montana The 4 control sheep receiving methylcholanthrene alone showed only normal tissue Periodic sacrifices are continuing at the present time

It seems evident that although the disease has probably been successfully transmitted in these experiments more than one year is required before clinical signs appear after inoculation This period cannot properly be termed a latent one since proliferative changes were observed in infected lungs after eight months to be replaced by the thirteenth month with the nodular lymphocytic congestion characteristic of pulmonary adenomatosis of Montana It was pointed out by Sigurdsson[16] that this is not a chronic disease but a progressive one Proliferative changes in the alveolar and bronchiolar cells actually may begin a few months after inoculation and the earliest stages of the Montana form of SPA closely resemble the earliest stages of the highly neoplastic Peruvian form Methylcholanthrene has shown no effect on the course of infection to date however the carcinogen was administered intraperitoneally and not at the site of virus action and in comparatively low dosage (100 mg per lamb)

Experiments are under way to effect transmission using various routes of administration intraperitoneal intratracheal intranasal and intrapulmonary inoculations have been made Material from apparently infected lungs of the first experiment has been used in an attempt at serial transmission and an additional group of lambs has been inoculated with new material from symptomatic Montana sheep At the present time no sheep from these groups have been sacrificed

Discussion

Of great interest is the relationship between infiltrative or inflammatory disease and adenomatous disease since these two aspects have been kept separate by custom yet each aspect in the case of the SPA complex can occur singly or in company with the other A possible parallel exists in fowl lymphomatosis in which experimental infection with one form may give rise to such conditions as erythroblastosis myeloblastosis or visceral or neural lymphomatosis all related serologically to the agents of the Rous sarcoma and the Fujinami myxosarcoma[61] In another case the formation of sarcomas or of salivary gland tumors in mice occurs in mice inoculated with the leukemia agent[67] The results imply that the agents involved are highly mutable maintaining a mixture of slightly differing viruses or that expression of disease depends to a large extent upon variations in cell or organ susceptibility in different host individuals

Either explanation serves to account for the mixed occurrence of infiltrative pneumonia and adenoma in sheep although both have been transmitted in relatively unmixed form *Maedi* (an infiltrative form) has been transmitted experimentally as has Icelandic PA (largely adenomatous) Only *Maedi* seems to occur as an unmixed condition and *Maedi* itself is found in areas overlapping that of the adenomatous Icelantic PA Thus it has not been

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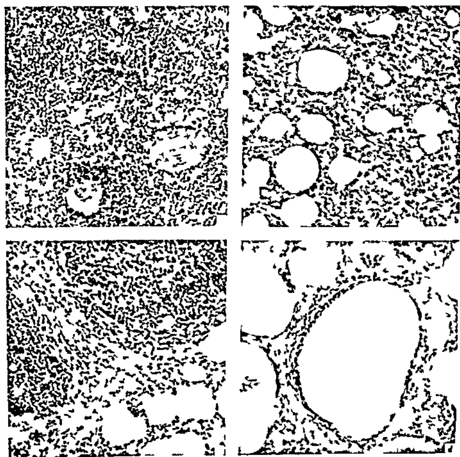


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FIGURE 7. Human pulmonary adenomatosis. Note the similarity to Icelandic PA (FIGURE 1b) and to *jaagsiekte* (FIGURE 2b) of sheep. (Specimen loaned by A. A. Liebow, Yale University School of Medicine, New Haven, Conn.) ×250.

demonstrated that either adenoma or infiltrative pneumonia is completely separable from the other, and the entire question as to whether these are different diseases may be meaningless for the present. In the end it may be necessary to resolve such differences by some arbitrary definition.

Many animals including man show conditions essentially similar to SPA both in histology and in clinical course. In man PA was first described in 1876 by Malassez[63] but judging from reports in the literature this disease has increased greatly in incidence during roughly the same period of time in which SPA has increased. It has been estimated that about 1 per cent of all human lung neoplasm is PA.[5] The disease in man differs from that in sheep almost solely in the incidence of metastasis, which is much higher in the former. Other features of PA in man are very similar to those encountered in SPA: dyspnea, rapid and shallow breathing, moist rales, emaciation, exhaustion, little or no fever in uncomplicated cases, and eventual termination in death over about the same period as in SPA unless supervened by early surgery in cases lacking bilateral dissemination. Histologically the diseases in animals and man are indistinguishable (FIGURE 7) with the qualification that in man no evidence has been brought forward to indicate that sterile progressive pneumonias are related to adenoma. The similarities between PA of man and sheep, however, have been observed by many workers.[4, 65]

Epidemiologically there is no reason to believe that PA in man is infectious. Irregularities in distribution are clearly accountable for by occupational environment. Thus silicosis may be followed by PA.[5]

Many bacterial and other conditions in man give rise to lung lesions that bear some resemblance to PA.[73] Proliferation in bovine lungs is said to follow nutritional irregularity or the ingestion of large amounts of certain minerals.[5] Although the role of nonspecific stimulation in the genesis of tumors is not well understood, it is difficult to account for the inexorable course of neoplastic diseases such as PA solely in terms of irritation, particularly when metastasis to distant organs occurs. Consequently, it appears that PA can

be considered an entity separate and distinguishable from pneumonia and metaplasia resulting from irritation

On the basis of its clinical progression and its general similarity to SPA C Bonne[64] and J Sims[51] have suggested the possibility of a viral origin for human IA Since those suggestions were made further study of SIA by workers in South Africa Iceland and the United States plus many recent experiments with animal neoplasia in general have revealed mechanisms of viral epidemiology that explain how a viral disease might be very difficult of detection as such as indeed is often the case Since many of these mechanisms tend to obscure the viral etiology of SIA they are of particular interest in consideration of the origins of neoplasms generally Some significant features of SPA may be described as follows

(1) The latency or slow progressive development of SPA has been well established experimentally by several groups of workers An asymptomatic period of several years between the time of infection and the appearance of clinical signs obviously tends to obscure infectiousness as a factor in development of the disease There is some reason to believe that natural SPA infection may occur early in life followed by a lapse of years before the disease is recognizable In studies on formalin killed vaccine against SIA Shirlaw[41] reports that vaccination after the first month of life is not considered effective in preventing the disease which subsequently develops in sheep several years old Infection of the young followed by long periods of latency is known to occur naturally in the case of the mouse mammary carcinoma [9] of fowl lymphomatosis [8] and of mouse leukemia [7]

(2) In these cases active virus can be recovered from the tissues of animals not showing the disease There is good reason to suspect that infection by the agents of SPA may occur at a higher rate than development of recognizable disease a factor also tending to obscure infectiousness Sigurdsson[16] has observed that lung lesions which seem to be abortive *Maedi* are found in healthy exposed sheep at incidences higher than those expected from the observed incidences of the diseases Marsh [3] has suggested that some early lesions regress without producing disease whereas Sigurdsson in experiments on *Maedi* believes that development of the disease is very slowly progressive If so many individuals should die from natural causes or be slaughtered before the disease appears

(3) The isolation of viruses giving rise to neoplastic disease is classically difficult to accomplish especially when inbred lines of the animal in question are not available for experiment In the case of SPA numerous methods for isolation of the causative agent have been attempted by several groups without success until the claim of Shirlaw was made [41] It is evident that failure to isolate the causative agent of any disease in no way excludes an infectious origin initial failure in the isolation of neoplastic agents is in fact more the rule than the exception

In the case of SPA infectiousness would doubtless not have been considered had the disease not occurred in epizootic form in certain areas In the case of PA in animals and in man although the incidence in the latter has increased somewhat in the last few decades the disease has not been observed in cycles

or epidemics, hence any attempt to extend the known infectious etiology of SPA to its counterpart in other hosts is highly speculative On the other hand information gained from the study of SPA should prompt closer examination of animal tumors generally, with the infectious viewpoint in mind as an etiological possibility

Acknowledgment

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(LOBAL I RIORITIFS I\ A\I\I \L-DISFASE CO\TROI

By \Iartin \I Kaplan

\\ orld He lth Organ at n Geneva S it-erland

In this paper I propose to discuss a pects and possibilities of certain human animal disease relationships These relationships constitute far more of a challenge than do the essentially technological solutions to the well-defined diseases

The permanent agencies concerned with animal disease control on an international scale are the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) with the I an American Sanitary Bureau (I ASB) which serves as the Regional Office for the Americas of WHO Their function in field operations coordination of research and international collaboration are described in official publications and I refer anyone interested to those documents The Office International des Epizooties (OIE) established in 1924 is another intergovernmental agency It meets annually has working commissions on special subjects with FAO and WHO participation and publishes and circulates official statistics on epizootic diseases The only other group that can perhaps squeeze into the category of permanency is the Point Four program of the United States government which is devoted to bilateral assistance agreements with various governments

Another area of WHO FAO effort is in food hygiene particularly with respect to meat and milk I pass over this aspect of our work even though considerable attention is given to this field because its interest lies in the application of primarily technological procedures (including the magic word radioactivity) to populations economically and socially unprepared as yet to receive them

The Major Diseases

Now a glance at the animal-disease problems having the greatest visible impact on public health and the economics of food production Here we shall use capsule summaries of the major di eases as they affect the less developed countries and as they may affect the more technically advanced ones

Foot and mouth disease The difficulties in the control of this malady may be summarized thus extreme communicability with such bizarre features as human and avian migrants as vectors at least seven distinct major types of virus with intratype variation and costly and cumbersome methods of control such as slaughter and ring vaccination with inactivated vaccines of short and often doubtful efficacy A light on the horizon appears to be the successful development of living attenuated strains for vaccination purposes the only practical answer for most countries apart from North America northern Europe and Oceania

Rinderpest In distinction to foot and mouth disease rinderpest is a rather

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problem but we are not optimistic about finding an early answer because relatively few laboratories are working in this field

Diseases of Less Importance

Now let us take a quick jump from these obvious diseases to more shadowy areas where future developments in human animal disease relationships are likely to be most interesting and fruitful Here we find that actual and potential zoonoses occupy only a part of these relationships while essentially noncommunicable entities begin to call for increased attention In keeping to my assignment however I shall deal only with the communicable diseases having an animal component known or suspected in their epidemiology To remain within the limits of the space at my disposal I shall use the device of question and no answer

Do the findings of *apparently* specific antibodies to the polio Coxsackie and adenoviruses in domestic animals and to canine distemper in humans have any significance in the epidemiological cycles of these diseases?

Do animal reservoirs play any part in the epidemiology of human influenza? Of toxoplasmosis?

What is the evolutionary history of the arthropod borne (arbor) viruses (or of any virus)?

To what extent are the orphan viruses being isolated from man and animals species specific? The psittacosis like organisms? What is their significance as disease producing entities?

Is rabies in nonvampire bats a new phenomenon or have these animals long been involved in the natural history of this disease? Are they of any importance in its epidemiology? Apart from vampire bats are there other asymptomatic carriers of the rabies virus in wildlife?

The common denominator of these questions is that we are dealing with viruses and their natural history a rich field for speculation with commensurate rewards in pragmatic achievement and intellectual satisfaction to those providing the keys to their solution

WHO is approaching these problems by taking advantage of its unusual opportunity to coordinate laboratory research efforts and field investigations This is illustrated by animal serologic studies on human influenza antibodies now under way in twenty five countries to determine whether animals are involved in the epidemiology of human influenza and by a world wide survey for Q fever prevalence recently completed In addition networks of laboratories are collaborating with WHO with respect to poliomyelitis treponematoses tuberculosis malaria and schistosomiasis apart from influenza rabies brucellosis leptospirosis and hydatidosis all mentioned previously

A Project of Interest

One further project as yet unimplemented may be worth mentioning It is clear to everyone that host parasite relationships are continuously changing and it is probable that new disease entities or unsuspected human animal disease relationships will evolve Until the present time one of the failures

easy disease to control Effective and inexpensive living attenuated vaccines have been developed and are available and even *rudimentary sanitary measures* can limit its spread The problem is how to use these tools under the difficult conditions prevailing in Africa and Asia but even on these continents the progress has been considerable for which we thank FAO for its energetic efforts In general, the same thing may be said about contagious pleuropneumonia hemorrhagic septicemia, and anthrax diseases of great destructiveness in these areas

Parasitic diseases The many ailments in this category unquestionably take the greatest economic toll Helminths flukes and blood parasites lead the roster Textbook recommendations for the control of these diseases often fail in the face of the realities of field conditions in underdeveloped countries The magnitude of the problems posed by these diseases, involving as they do the social organization and economic structure of these countries, is such as to make impossible any early solution

Brucellosis and leptospirosis These two diseases illustrate the close working relationship between WHO and FAO as they deal with zoonoses problems of economic and public health importance

The major problem at this time with respect to brucellosis lies in the development and application of a vaccine effective in sheep and goats During the past five years WHO and FAO have coordinated and supported research in this field and results are encouraging In October 1957 the joint FAO WHO Expert Committee on Brucellosis will have met in Lima Peru to evaluate the experimental work carried out on brucellosis in the fifteen FAO-WHO brucellosis centers located in different parts of the world and the report of this committee should be useful as a guide to our member countries

In leptospirosis WHO and FAO have concentrated their efforts on typing procedures with reference to *Leptospira* and in developing standard diagnostic tests that can be used without too much difficulty in hospitals and laboratories in the routine diagnosis of this disease in man and animals This work is being carried out through six WHO FAO Reference Leptospirosis Laboratories one each in Australia Holland Italy Japan the United Kingdom and the United States

Rabies Most of you are aware of the considerable attention WHO has given to rabies with respect to the improvement of vaccines investigations of hyperimmune serum in prophylaxis and in wildlife studies Perhaps one of the greatest obstacles in the way of advances in laboratory research connected with rabies has been the inability to cultivate the virus on tissue culture to any satisfactory degree

Hydatidosis This disease deserves brief mention because of the extent of human illness it produces as well as the very great economic losses it causes in livestock products in South America in the Mediterranean area and in Oceania Effective control of this disease cannot be achieved under conditions found in most affected areas until a better drug than arecoline is found for the treatment of dogs infested with the *Echinococcus* tapeworm and until more practical ovacides than creolin are developed We are encouraging research on this

problem but we are not optimistic about finding an early answer because relatively few laboratories are working in this field

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in studies on communicable diseases has been our inability to follow the progression of disease in geographical areas at different points of time This failure cannot be attributed to human oversight but rather to the limited opportunities and tools available for instituting appropriate studies While conditions are better in this connection it will be many years before there will be sufficient facilities to cope with the many problems associated with progression parameters in the epidemiology of communicable diseases Nevertheless in the meantime certain steps are possible that would no doubt be of invaluable assistance later when more detailed studies will be feasible This thought underlines the reasoning behind projected work in epidemiological studies based on serology Briefly it is planned to arrange for serum specimens to be taken from different age groups in human beings and animals in various parts of the world within a given span of time to catalogue these specimens and to store them in a refrigerated or freeze dried state for examination perhaps many years later Thus where a particular study will be undertaken on one or more diseases serum specimens from the human and animal population of a geographical area of interest would be available for examination by qualified laboratories

Such work can be done uniquely by an organization such as WHO with the collaboration of FAO and should be of immense benefit in improving our knowledge in control efforts of communicable diseases both in the near and more distant future WHO s role would be principally that of a coordinator for it should be stressed that the real contribution and effort would come from the collaborating laboratories and scientists throughout the world who have so generously contributed their services and facilities in the past and who we do not doubt will continue to do so

THE PAN AMERICAN ZOONOSIS CENTER

By Benjamin D Blood
Pan American Zoonoses Center Azul Argentina

The Pan American Zoonoses Center located in Azul province of Buenos Aires Argentina is an international institution with the basic objective of promoting and strengthening activities against the zoonoses in the countries of the Americas The center serves these countries in the education and training of professional and lay technical personnel in techniques and methods to be used in combating diseases transmitted between animals and man It stimulates zoonoses control and eradication campaigns in the different countries and coordinates such campaigns on an international basis Its staff is available for consultation on problems and activities concerned with those diseases It conducts research with the aim of improving diagnosis and control of the zoonoses and it promotes aids and coordinates such research in governmental and private institutions throughout the Americas It works toward standardization of diagnostic methods and procedures for making and testing vaccines sera and other biological products used for the zoonotic diseases It prepares and disseminates information about those diseases All of these services are aimed at helping the people of the Americas with the understanding and support of their governments to reduce the social and economic burden caused by uncontrolled diseases common to people and animals

The Pan American Zoonoses Center was formally established on August 10 1956 with the signing of the agreement between the government of Argentina as host country and the Pan American Sanitary Bureau (PASB) Regional Office of the World Health Organization (WHO) Washington D C as the responsible international agency The first staff members were assigned late in that year and the work of organizing and installing the center was begun The staff has since been enlarged according to definite schedule until at the present time there are four international officials and sixteen local employees at their posts The number of local employees will have increased to at least twenty by the end of 1957

Location and Facilities

A number of countries expressed interest in becoming host to the center Upon careful study of each of the different proposals it was judged that the facilities offered in Argentina were the most adequate

Azul a city of about 25 000 population located 185 miles south of Buenos Aires the Argentine capital is situated in an important livestock raising region The cooperation of the medical and veterinary professions livestock breeders hospitals municipal authorities and other local groups is an important asset of this international institution

The building provided by the Argentine government is very well suited for housing the Pan American Zoonoses Center Although built for another

purpose it was never occupied until transferred for its current use It is a two story structure with space adequate for present needs in laboratories offices classrooms and library all of which are now complete or in final stages of remodeling There is land remaining on either side for additional construction as needed The host government provides the funds required for public utility services and for the maintenance and servicing of the building and has agreed to finance new construction as necessary

As an adjunct to the central facilities the Argentine government has also purchased a 370-acre farm annex for the exclusive use of the center This annex is less than three miles from the central site and provides the location for isolation units holding pens, the laboratory animal colony, and supporting services

Administration

The Pan American Zoonoses Center was created in response to desires of the American governments as expressed both individually and collectively in several international meetings political as well as and technical Its financial and material support are presently derived from three sources the host government the Pan American Sanitary Bureau and the United Nations Technical Assistance Program Negotiations are now in progress to broaden the financial structure and to assure its operation on a permanent basis

The center is sponsored and administered by the PASB and is subject to the rules regulations and procedures of that organization Other agencies interested in the objectives and activities of the center may participate in it by accepting the terms of the agreement by which it was established

The permanent technical staff members including veterinarians physicans and members of allied professions are international employees that is their posts are supported with international funds In addition specialists in various aspects of zoonosology are under contract for limited periods and are paid with international or special funds The permanent supporting staff (that is the lay and nonskilled personnel) is recruited locally in Argentina and at this time is supported by funds contributed by the host government

Functions and Operations

The diseases classified as zoonoses are so vast in number that the center will not be able at least initially to give due attention to all of them Priorities are being established in accordance with the relative impact on human welfare and the geographical extension of the different zoonotic diseases in the Americas Accordingly brucellosis hydatidosis and rabies are being given major attention followed by virus encephalitides anthrax leptospirosis tuberculosis psittacosis trichinosis and salmonellosis This does not mean that the remaining diseases are to be ignored completely since the center is intended to be a clearinghouse for information on all the zoonoses Furthermore the priorities may be revised from time to time to coincide with epidemiological/epizootiological changes and recognized needs of the countries

The center is not intended to replace government zoonoses activities nor even to compete with them Its role is to complement those activities by

providing services that the individual countries have not found it possible to develop as well as to coordinate those activities internationally The functions and services of the center are essentially education and training consultation and coordination laboratory services demonstrations research and information

Education and training The educational and training activities are probably the most important part of the work of the center The lack of adequate numbers of well trained personnel both professional workers and lay assistants is a problem recognized throughout the Americas although the need is somewhat more acute in some countries than in others It is obviously impossible to carry out successful diagnostic field control eradication treatment or research work without a qualified staff In order to help provide the personnel required the center offers a variety of training courses and opportunity for specialized study

Training methods will make maximum use of demonstration and of trainee participation Lectures are supplemented with audio-visual aids laboratory work is arranged so that each trainee actually practices the specific techniques under study and field demonstrations make it possible for participants to become throughly familiar with methods for applying recognized measures of disease control

Fellowships for training at the center are provided by individual governments and by PASB and WHO they will undoubtedly also be provided by other agencies Training is offered on both short term and long term programs Special courses seminars and workshops of from one to twelve weeks duration are being scheduled with attention given to special topics or fields of work The individuals accepted for such training are those who already have the necessary professional or technical preparation to continue

One course that will be repeated on an annual basis is for a three to four week period devoted to zoonoses control for recent graduates from schools of public health The course will be for veterinarians as well as for physicians preparing for epidemiological or rural health assignments Other courses to be held are for example on the laboratory procedures in brucellosis including diagnosis and vaccine production on the diagnosis of rabies and on control methods for hydatidosis An annual two-month course will be offered for training nonprofessional field assistants for antizoonoses work Other courses and seminars will be held as needs and possibilities arise

Long term training is offered for individuals to undertake special study in one or more of the many phases of work with zoonoses These individuals are expected to remain at the center for at least 6 to 12 months for postgraduate specialization and research It is anticipated that this type of training will be developed in many cases in cooperation with universities

It is highly desirable that the Pan American Zoonoses Center operate in close cooperation with colleges and universities Although the exact patterns for such cooperation remain to be developed there are excellent possibilities for the center to work with schools of public health medicine and veterinary medicine at both the undergraduate and postgraduate levels of study One of the several forms that collaborations might take would be for students to

perform their field (thesis) work at the Zoonoses Center after having completed the required formal courses at a university

The educational activities of the center also include the collection and distribution of audiovisual materials for use in programs dealing with the zoonoses Such materials will in addition be developed at the center and they also will be produced there on a limited scale when not already available

Consultation and coordination Upon request from the government concerned the center is prepared to send members of its staff to visit countries in a consultative capacity on matters related to the zoonoses and their control In addition consultation services are provided through correspondence Special attention is being given to the development in each country of the mechanism necessary for planning and operating control programs for the zoonoses Such mechanisms based on the concept of united effort should provide for coordination and cooperation between public health and animal sanitation agencies with maximum participation of the community Visits by consultants also provide the opportunity for international coordination of control and eradication programs

Laboratory services These services include reference diagnosis standardization of antigens vaccines the production of sera and other biological products confirmatory testing of such products for potency and safety, and the distribution of strains of viruses and bacteria for production or challenge purposes and of standard antigens vaccines and sera for use in check testing

The center will operate a model colony of laboratory animals that will serve for training demonstration and study purposes as well as for supplying foundation stock for other institutions

Demonstrations The center will utilize field campaigns against various zoonoses in pursuance of its functions Trainees are taken to the site of the work and actually participate in zoonoses control programs Existing anti zoonoses programs of the city of Azul and of the host country (at both provincial and national levels) serve for such demonstration purposes Demonstration work will also include the development of special control programs for various zoonoses in different parts of the Americas the number type and extent of such programs being dependent upon the needs capabilities and desires of the country concerned

Research Research at the center is of an applied nature that is the evaluation of a vaccine under certain field conditions the practical application of a diagnostic procedure the assessment of drugs for their antiparasitic effect and similar projects Surveys and epidemiological epizootiological studies will be undertaken with respect to certain of the zoonoses Also included under research is the collection and processing of statistical data on the occurrence and over all importance of the diseases in man and animals

The needs and opportunities for research in connection with the zoonoses are vast and the center itself can be expected to cover only a very small part of them It is thus quite evident that public and private institutions in all countries must continue and intensify their research work in this field It is a responsibility of the center to stimulate and coordinate such work

Information Facilities are being developed so that the center may serve

as the source of both technical and popular information on the zoonoses Library service will be an important activity with microfilms and photocopies of technical references made available to investigators and control officials Special priority is being given to the collection and cataloguing of books periodicals bulletins and reports on diseases common to man and animals

The film library will be made as complete as possible with a collection of all available films film strips and slide sets concerning the zoonoses and their control When possible new films will be produced on subjects on which no suitable material already exists

A periodic information bulletin is to be published this will contain abstracts of the world literature on zoonotic diseases as well as brief references to the latest developments in this field as they may occur in the Americas and elsewhere

A collection of bulletins pamphlets illustrated booklets posters exhibits and other items used with success in the public education aspects of zoonoses control is maintained for demonstration and loan New material of this type will be prepared at the center as needs and resources permit

Summary

The recently established Pan American Zoonoses Center is an international institution sponsored by the Pan American Sanitary Organization (Regional Office of the World Health Organization) and is dedicated to promoting and strengthening activities against the zoonoses in the countries of the Americas Its services are available to health departments agriculture departments educational institutions and other agencies having an interest in zoonoses the diseases naturally transmitted between animals and man

The center which has its central site in Azul Argentina is designed to serve all of the Americas in the education and training of professional and lay technical personnel in techniques and methods to be used in attacking the zoonoses It will also (1) conduct research with the aim of improving diagnosis epizootiological-epidemiological knowledge and control procedures (2) promote aid and coordinate such research in governmental and private institutions throughout the Americas (3) work toward the standardization of diagnostic methods and procedures for making and testing vaccines sera antigens and other biological products (4) prepare and disseminate information on the zoonotic diseases and (5) develop field demonstration activities in accordance with the needs and desires of participating countries

The principal aim of the center is to promote and strengthen governmental activities against the zoonoses in the countries of the Americas All of its activities whether in the field of training demonstration research information or coordination have been planned for that purpose It is fully recognized that this objective can be reached only insofar as there are sound technical activities and services in each country dedicated to research control and eradication of diseases common to man and animals

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Demonstrations The center will utilize field campaigns against various zoonoses in pursuance of its functions Trainees are taken to the site of the work and actually participate in zoonoses control programs Existing antizoonoses programs of the city of Azul and of the host country (at both provincial and national levels) serve for such demonstration purposes Demonstration work will also include the development of special control programs for various zoonoses in different parts of the Americas the number type and extent of such programs being dependent upon the needs capabilities and desires of the country concerned

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The needs and opportunities for research in connection with the zoonoses are vast and the center itself can be expected to cover only a very small part of them It is thus quite evident that public and private institutions in all countries must continue and intensify their research work in this field It is a responsibility of the center to stimulate and coordinate such work

Information Facilities are being developed so that the center may serve

Among the basic principles of epidemiology is the concept of reservoirs of infection It is always desirable to have our problems appear in simple form so that understanding them easily we may achieve simple solutions In the area of the zoonoses however the relationship of source and/or reservoir of infection is seldom direct and rarely simple To paraphrase Cameron s words the complicated life cycles of parasites their facilitation of interchange between alternative hosts by close association their varying pathogenicity dependent upon recency of host acquisition the favorability of the host environment for multiplication the forms of the invading parasite their essential or alternate modes of transmission which in turn are dependent upon their ability to survive in transit or in contiguous species and the phylogenetic or physiologic kinship of the several hosts—all complicate animal human disease ecology and make our search for reservoirs of infection both frustrating and revealing It is obviously this continuous challenge coupled with the necessities of human health and economy that leads to our explorations in the complex areas of the viral encephalitides rabies leptospiroses salmonelloses plague and typhus to name but a few

If I were asked to select the most ecologically complicated entity from among these my task would not be simple Whether it be the phenomenon of wintering over of encephalitis viruses the reasons for geographical differences in endemic and epidemic vectorial spread of these viruses the significance of bat rabies to human health the multiplicity of sources of salmonella or the reservoirs of plague the complexity of these problems is exceeded only by our ignorance of them Deep inroads however have been made Hess and Holden in their paper on The Natural History of the Arthropod Borne Encephalitides in the United States and Chamberlain in his contribution on Vector Relationships of the Arthropod Borne Encephalitides in North America have presented certain areas of agreement that help substantially to clarify some aspects of this complex ecologic problem They are apparently agreed on birds as the most common vertebrate hosts on *Culex tarsalis* as both the endemic and epidemic vector of western equine encephalitis (WEE) and St Louis encephalitis (SLE) in the western United States on *separate* endemic and epidemic vectors for WEE SLE and eastern equine encephalitis (EEE) in the eastern part and on *Culex pipiens* as the vector of SLE in the east and of urban SLE in the west In addition Hess and Holden have presented evidence that both birds and hibernating mosquitoes are potential overwintering mechanisms for WEE and SLE viruses Speculating on the relative infrequency of encephalitis epidemics in the east these investigators have postulated that natural environmental factors must concurrently provide optimum conditions both for vector breeding and longevity and that irrigation practices in the west provide these optimum conditions Kissling in his paper on Host Relationship of the Arthropod Borne Encephalitides has helped us to visualize the determinants of geographical spread among reservoir hosts by categorizing the encephalitis viruses of group A according to their abilities to propagate in certain species with WEE propagating in birds Venezuelan equine encephalitis (VEE) in mammals and EEE intermediate with birds however affording the best viremia levels for transmission

Summary

By Leonard M Schuman

School of Public Health University of Minnesota Minneapolis Minn

The role of summarizer of a monograph as diversified in subject matter as this is a difficult one especially when the entire tone and orientation of the work have been so ably keynoted by the introductory presentation of J D Martin A summarizer, furthermore is constantly subject to the risk of routine repetition and the pronouncement of impressions already obvious to the reader Despite the hazards of such a role it offers the humble privilege of pointing out the highlights of the monograph attempting to assess its impact and interjecting bits of philosophy and speculation while distilling the essential truths from the matrix of contributions

Although the admirable presentations of each of the contributors guarantee the value of this publication a significant share of the credit must go to the conference chairman and the members of his organizing committee for adroitly bringing together the talents of the several disciplines—laboratory epidemiological, clinical, and administrative—of human and veterinary medicine From this observer s point of view the subject matter is happily balanced for topics of practical and continuing importance on the one hand and provocative newer knowledge at the frontiers of the life sciences on the other

The very title of this publication conjures up an image of human ecology, serving to efface the customary categorical but nonetheless artificial distinctions between human and animal disease It immediately denies the treatment of human and animal diseases as isolates and serves to set the pace for an approach to the complex interdependence of all living matter in a common environment From the papers by Hsiung and Melnick on Orphan Viruses of Man and Animals and by M Klein on The Significance of Human Antiviral Neutralizing Substances in Animal Sera to the philosophically stimulating presentation by Cameron on Parasites of Animals and Human Disease we are even more fully impressed with the underlying interrelationships of basic living matter in the form of etiological agents of disease despite the emerging host specificity of the parasites The contribution by Hsiung and Melnick not only reiterates these relationships but perforce broadens our concern with respect to the potentialities of nonhuman sources of orphan viruses Now that these original orphans have found a haven of security in the form of human disease their importance takes on increasing significance Klein s contribution is of a similar character philosophically for the provoking presence of human antiviral neutralizing substances in the sera of animals in which the related disease states apparently do not exist and from which the specific antigens have not been isolated leads to wholesome speculation Whether ultimately a common antigen is discovered to explain these antibodies or antibody like substances or a mechanism of formation is elucidated to revolutionize our concepts of antigen antibody relationships the pursuit of this investigation bears great promise in furthering our as yet inadequate understanding of animal human disease ecology

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Although neither as complex in its host parasite relationship nor as productive of human epidemics as the viral encephalitides the leptospiroses represent a group of etiologically related diseases with a multiplicity not only of reservoirs and sources but of clinical syndromes in animals and man Stockard and Woodward s contributions to our clinical understanding in their paper Leptospirosis Infections in Man' make it obvious that despite their relatively infrequent occurrence in man in outbreak form the leptospiroses have so broad a spectrum of clinical features (ranging from short term pyrexias in influenza like illnesses through central nervous system infections simulating nonparalytic polio to fulminating disease resembling fatal cases of yellow fever) that coupled with the ubiquity of animal reservoirs these etiologically definitive entities must constantly be considered in the differential diagnosis of human disease

The dissertation on Animal Reservoirs of Leptospires by Babudieri reminds us that efficient carrier states are produced only when a satisfactory equilibrium between the leptospire and the host has been established He notes that while domestic animals and rodents are particularly important reservoirs of infection for man, pathogenic leptospires recently have been isolated from migratory birds Babudieri re emphasizes the need for concurrent conjoint and integrated epizootiological and epidemiological investigations In their paper on Epidemiological Patterns of Leptospirosis Galton Menges and Steele reiterated the universality of these infections among several animal species their pathogenicity for man and the role of the animal carrier Their presentation of epidemiological patterns in the United States and the human epidemic potential inherent in existing serotypes as well as the discovery of new sylvatic hosts serve to augment this observer s appeal for a concerted multidisciplinary approach to the problem

Among the earliest recognized zoonoses rabies has continued to constitute a serious threat to human populations As such and because of recent developments both in the epidemiology and prophylaxis of the disease the prominence given it in this monograph is justified In reviewing Recent Developments in the Epidemiology of Rabies Tierkel has pointed to the decline in canine rabies as evidence of the success of control measures applied but he views with concern the increase in the disease among sylvatic vectors Although it is difficult to separate reports based on field investigations and research or stimulated by animal morbidity reporting systems from real increases in incidence the growing awareness of ever unfolding wildlife reservoirs is salutary to the definition of the problem despite its growing complexity Tierkel thus correctly presented the need for expanding investigational activities in the epidemiology of the disease in wildlife

Of provocative interest was the paper Bat Rabies Experimental Host Transmission Studies by Burns This investigator s experiments on animal transmission of bat rabies virus by induced association with resultant inability to demonstrate transmission from experimentally and naturally infected bats to monkeys guinea pigs and white mice as well as the lack of evidence of clinical recovery or of the development of efficient carrier states among inoculated insectivorous bats will provoke I am sure a flurry of laboratory and field

investigations of this potential reservoir of infection Burns admonition to treat bat bites as potential exposures to rabies until more extensive studies are available is well made

Salmonellosis is rarely discussed in works on animal diseases related to human health Consequently interest in this disease appears to be confined to the general public which often fails to avail itself of the care of the practicing physician who in turn rarely undertakes the etiological definition of diarrheas when patients do come for treatment and of the health officers who all too often make ineffectual gestures toward control measures It is therefore noteworthy that no less an expert than Edwards has presented his paper Salmonellosis Observations on Incidence and Control in this volume Presenting available information on cases and outbreaks in man this investigator very properly calls attention to the gross inadequacy of these data as indicators of true incidence and points to the similarity of serotypes in human and animal populations He reiterates the frequently indigenous character of salmonellae in animal foods and appeals to medical and paramedical personnel involved in human and animal health to institute effective measures for the control of infections in animals

Despite the rarity of even sporadic cases of plague in the United States today the existence of heavily infected reservoirs among sylvatic rodents denotes our failure thus far to control sylvatic plague and to remove this potential threat to human populations As worthy a challenge to ecologic investigation as any disease sylvatic plague embodies complex relationships among the reservoir host the ectoparasitic vector and the bacterial parasite relationships that have been studied very inadequately Kartman and his co workers in their paper on New Knowledge on the Ecology of Sylvatic Plague indicate studies in progress that will remedy these inadequacies in large measure Of highly significant import is their finding that a replacement of emphasis on specific reservoirs must be made Whereas prior to 1950 ground squirrels and prairie dogs received prime emphasis recent years have revealed that field mice principally of the species *Microtus* are the important reservoir The high infection rates of *Microtus* fleas their prevalence in the field and their high efficiency as vectors make the species *Microtus* a primary source of plague transmission Kartman emphasized the significance of this threat to human populations by pointing to the suburban habitat of *Microtus* and its coexistence with rats in dumps and parks and around homes where flea transfer to domestic rodents can occur

Murine typhus has also presented problems in ecology although they are not nearly as complex as those of plague Pratt in The Changing Picture of Murine Typhus in the United States reviewed the reported incidence of the disease in two four year periods noting an increase from 1941 to 1944 and a marked decrease from 1953 to 1956 With all due respect for the sanitation programs that undoubtedly contributed to the control of rodents and their ectoparasites we cannot overlook the possibility of natural downward trends in rat and flea populations or the acceleration of the decline in the disease by more exact diagnosis with laboratory confirmation stimulated by the murine typhus surveillance program of the Communicable Disease Center Atlanta Ga

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In this same respect Koprowski who was given a most difficult task in finding Counterparts of Human Viral Disease in Animals stresses the need for expanded research in the area of comparative pathology and epidemiology warning us however to avoid equating the unknown with the unknown and overlooking species differences in clinical response Certainly his curious finding of poliovirus Type I in calf feces should be explored thoroughly

Thus far I have dealt with the epidemiological considerations of animal hosts reservoirs and agents of disease discussed or implied in the several presentations However animal diseases that do not necessarily occur in man may *have equal if subtle importance in human ecology I refer now to a group of* papers dealing with the study of purely animal diseases for their value as research tools in eliciting pathogenetic truths common to human disease Pollard s Comparative Studies on Viral Hepatitis in Animals and in Man deals with the use of mouse hepatitis virus as a possible prototype of the human disease in lieu of work with the human virus which is not transmissible to other animals and which thus far has not been grown in tissue culture This work is illustrative of the methodology necessary to circumvent obstacles in the study of human disease

It is gratifying that this monograph does not confine itself entirely to *acute* communicable disease but seeks to explore the comparative pathology of certain chronic diseases or chronic sequelae to infectious disease in animals that show histological configurations similar to those of important diseases in man The contributions of Sikes on A Comparison of Rheumatoid Like Arthritis in Swine and Rheumatoid Arthritis in Man and of Duran Reynals and Rafferty on The Pulmonary Adenomatosis Complex in Sheep are cases in point An approach via comparative pathology might not reveal identical underlying etiologies for the counterparts of human disease but it should yield information of great value in the study of neoplastic and other chronic diseases of man

I should not overlook the area of diagnostic tests in the zoonoses Gochenour and his co-workers in reviewing the Laboratory Diagnosis of Leptospirosis presented the merits and disadvantages of the several tests employed In their hands the killed antigen agglutination test gave results identical to those with live antigen In discussing more recently developed tests these investigators noted excellent correlation of the capillary tube test of Stoenner with live antigen More nearly genus-specific antigens such as the sonic vibrated complement fixation (CF) tests of Randall the erythrocytic sensitizing test of Chang and the hemolytic test of Cox requiring but a limited number of antigens yielded only fair correlation with agglutination tests Gochenour and his collaborators expressed the need for a more rapid means of recovering leptospires and for a simple inexpensive sensitive genus-specific test for local laboratories It is gratifying however that work in this direction is proceeding

The paper on "Recent Developments in Animal Ringworm and Their Public Health Implications" by William Kaplan and his co-workers was, in this reviewer's opinion, very welcome. The epidemiology of mycotic infections is an all too neglected field and there is great need for inquiry and important contributions in this area. Kaplan's proposal for a functional classification of dermatophytes based on epidemiological relationships and comprising three groups—anthropophilic, zoophilic, and geophilic—appears to be a practical one.

If the public health importance of a disease were dependent upon the completeness of our understanding of its epidemiology, then a number of diseases presently engaging our intense attention would be relegated to the unimportant. The lack of attention to a number of diseases that are common to animals and man is frustrating at times. All too frequently the lack of awareness of a disease determines that it shall fail of recognition and intensive study. In "Listeriosis: A Potential Public Health Problem," Winn and his co-workers assert this for a disease whose ubiquitous agent is well known, its pathology in animals well documented in veterinary medical science, but whose epidemiology is most inadequately understood. To me this represents, primarily, a failure to apply an interdisciplinary approach. It is my hope that Winn's presentation will stimulate our thinking in this direction.

It is a rare disease indeed for which the epidemiology is relatively completely known. In man, the several modes of disease transmission have not been satisfactorily worked out and their natural histories are poorly understood. The value of the contribution of Welsh and his co-workers in their extremely fine paper on the "Air-Borne Transmission of Q Fever: The Role of Parturition in the Generation of Infective Aerosols" lies not only in the excellent review material presented, but in the original work described, which adds to our understanding of the modes of transmission of the disease in the area of air-borne infection. The experimental work of these investigators on the production of aerosols during the parturition of inoculated sheep and the persistence of these aerosols in the environment is classic in form, and once again it emphasizes the contribution that animal disease study can make to human epidemiology.

In dealing with animal reservoirs of human disease, we customarily expect disease counterparts in the animal host or at least serologic evidence of infection to denote a host-parasite state of equilibrium. Cat scratch fever, according to Prier, is geographically widespread, quite common, presents a typical clinical picture, and is expected to increase in incidence because of man's firm association with these domestic pets. Of special epidemiological interest is the fact that no definite clinical disease in the animal host has been recognized.

Although Hanson and Brandly, in their discussion of "Newcastle Disease," minimize its role as an occupational hazard among poultry producers and handlers and speak of its indirect impact on human economy principally as an avian disease, to the epidemiologist the rare occurrence of a disease is equal in importance to a wide-scale outbreak in the attempt to arrive at a more complete understanding of the agent-host-environment relationship. Not long ago Newcastle disease could have been included in the list of animal diseases that are known not to infect man. It is in this philosophical vein that Shahan and

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symptom of our failure to achieve a safe and efficient vaccine for humans Serving not as a substitute but in a sense as an adjuvant to vaccine hyperimmune serum augments our treatment but fails to obviate the hazard of reactions It was inevitable then that chick embryo adapted living virus vaccines should be applied to volunteer human groups

Although at this moment field experiments on seroimmunity reveal phenolized vaccines to be most efficient the demonstrations by Kox in collaboration with the World Health Organization (WHO) of the practicality of pre bite long term immunization by primary and booster vaccinations with high egg passage Flury vaccines and the rapid antibody response in Pasteur treatment conditioned individuals to boosters of the avianized vaccine hold great promise for human prophylaxis Certainly their implications for persons at high and relatively constant risk is quite obvious when we consider the reaction potential in repeated immunizations effected by Pasteur techniques

Brucellosis which has suffered an unwarranted loss of emphasis in many conferences on the zoonoses is not neglected in this monograph Despite the optimism expressed by Mingle in his paper on Brucellosis in Livestock Control and Eradication with respect to bovine disease and despite his prediction that there will be less than 1 per cent infected cattle in the United States by 1960 a note of pessimism was injected by McCullough who although he noted the great strides made in control of bovine brucellosis stressed the lack of attention paid to swine brucellosis and the virtual lack of efforts toward controlling it When one considers the fact that today this disease is found predominantly among packing house workers and others who handle animals one must be concerned over the possibility that a significant level of human brucellosis will be maintained far short of the irreducible minimum (not to mention complete eradication) even after bovine brucellosis is virtually eradicated

The complexity that animal human disease relationships may assume and the gross alterations in epidemiology and control applications that may be induced by economic factors is well illustrated by anthrax In their paper Industrial Anthrax Brachman and Fekety have quite competently demonstrated the shifting sources of infection through improvement of animal health and have described the impact of war which changes the source of contaminated raw materials and technological advances such as the development of plastics and synthetic fibers that completely remove certain animal materials from the list of hazards The bulk of anthrax today is industrial rather than agricultural and in industry goat hair processing presently constitutes the principal source Here is an admirable example of the need for constant epidemiological appraisal of a problem so that we may remain poised for attack from any quarter Of additional significance in Brachman and Fekety s contribution is their interesting approach to the question of achieving human immunity by prolonged exposure The data of these investigators suggest that such immunity does not develop

Bovine tuberculosis has received little attention in more recent years chiefly because of the tremendous success of its control program In his contribution on The Public Health Importance of Animal Tuberculosis Anderson has cited the gains made by the now historic program of tuberculin testing of herds

The detection of ornithosis in domestic fowl is grossly inadequate at best Benedict exploring the indirect CF test and modifications of the direct CF test *along with an intradermal test found 70 to 85 per cent agreement between the* allergic and serologic tests and proposed the application of the intradermal test as the most practicable for ornithosis surveys It is hoped that field tests of the method will provide us with the necessary tool for augmenting control of this economically devastating and at times human epidemic disease

The ultimate goal of laboratory workers clinicians and epidemiologists is the control of disease This monograph includes reports of satisfying achievements in several disease areas Sussman Cohen and their co workers applied EEE vaccine to flocks of pheasants in New Jersey during the 1956 epizootic *with surprisingly good results Of significance is the fact that appreciably less* than 100 per cent vaccination of a flock during an outbreak will provide reasonably high protection for the flock as a whole with evidence that nonvaccinates in a partially treated flock also enjoy relative protection Applied prior to an outbreak partial treatment of a flock gave even higher protection rates To an epidemiologist this work represents more than the achievement of a practical tool to control an animal disease that is both of economic importance and a potential hazard to man In it I can see a striking example of experimental epidemiology in the field a sadly neglected area of inquiry since the pioneering *work of Greenwood Furthermore the study on partial flock immunization* represents a direct counterpart of human population immunization phenomena with the significant exception that the latter have involved conjecture more often than analytic observation As such the pheasant study represents an epidemiological model with aspects that may throw light upon human epidemic dynamics

In turkey ornithosis Delaplane succeeded in establishing prophylactic levels of chlortetracycline with apparent total suppression of virus when dosage of more than 200 gm per ton of feed for two weeks or 200 gm per ton for three weeks was employed Furazolidone was found to be ineffective Of collateral epidemiological interest was Delaplane s finding that virus was apparently not transmitted via the egg even during the viremic stage when infection potential is highest The application of these observations may well augment our efforts at control despite our lack of an active immunizing agent Its indirect significance in human disease prevention is quite obvious when we realize the epidemic potential of this disease reservoir

In charting the development of prophylactic measures for the control of rabies in domestic animals Kaeberle very properly stressed the fact that the continuous attempts to develop attenuated living virus vaccines were indicative of the inadequacy and disadvantages of vaccines of nervous tissue origin The effectiveness of avianized low egg passage Flury strain vaccine has now been well demonstrated for dogs and the application of high egg passage vaccines to cattle is a recent bright spot in our progress

What has been said of rabies vaccines for domestic animals applies equally if not more so to human prophylaxis Here a sword of Damocles has hung on a finer thread and the formidable specter of treatment reactions has plagued us since the days of Pasteur The advent of hyperimmune serum was another

symptom of our failure to achieve a safe and efficient vaccine for humans Serving not as a substitute but in a sense as an adjuvant to vaccine hyperimmune serum augments our treatment but fails to obviate the hazard of reactions It was inevitable then that chick embryo-adapted living virus vaccines should be applied to volunteer human groups

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slaughter of reactors and tracing of sources of infection The relatively rare cases of bovine tuberculosis in humans speak well not only for the success of the control program but unfortunately also connote our failure to eradicate this disease completely It must be remembered that Modified Tuberculosis Free Area Certification still implies a maximum of one half of 1 per cent individual animal infection It is the history of human populations that complacency leads to apathy and apathy to disaster Our attitude is unfortunately tied to our definition of the word problem' To some this means only clinical disease either in man or in animal reservoirs to me (and I hope to many others) it means a great deal more for it includes the potential of individual and epidemic hazard even after mass control has been achieved It matters not whether it be diphtheria or bovine tuberculosis for it is the very essence of life that new generations without immunity without protection and without knowledge shall be born Thus eternal vigilance directed toward unrelenting maintenance of control becomes the second phase of every problem of disease control bovine tuberculosis is no exception

The interdependence of animals and man and their ecological relationships in disease can lead literally to rather inhuman applications Hempy in his fine summary of 'Animal Disease and Biological Warfare presented the general philosophy of the use of disease agents in biological warfare as a method of reducing a nation's military effectiveness He stressed the fact that aerosols are readily deliverable by any country and that there are no secrets among nations relative to potential infectious agents of disease Because of the attributes that such microorganisms must possess to be effective agents in warfare the number available is actually limited These attributes include high infectivity simple dissemination, ease of production inefficiency in the production of solid immunity high lethality (or at least pathogenicity) and mutability toward resistance to chemotherapy In addition to the direct aerosol application of suitable zoonotic microorganisms to human populations the application of aerosols to crops and food animals could result in fatal reduction of food supplies Hempy emphasized that our preventive techniques are an advantage in this area but they certainly provide no cause for complacency More agents of effective active immunization both for man and for animals are needed Hempy s precise delineation of the problem should serve as a stimulus in this vital field

This monograph includes problems not only of national but of global importance For the vast number of diseases of man whether of human or animal origin it has frequently been stated that as long as a disease exists anywhere in the world it remains a constant threat to populations in more favorable positions The underprivileged countries of the world with their more numerous problems of animal disease of direct or indirect importance to human health and food availability must be treated altruistically and with humanitarianism These were the implications of Martin Kaplan s discussion of Global Priorities in Animal Disease Control The aggressive and well conceived programs of animal-disease control of the World Health Organization (WHO) in collaboration with the Food and Agriculture Organization (FAO) and the Office International des Epizooties (OIE) should achieve not

only the objectives of animal and human health but should provide valuable field data contributory to even newer epidemiological knowledge and control techniques In this regard it is quite admirable that the integration of the several disciplines involved in animal and human health has progressed to the point that animal sera will be collected and stored for future titrations to determine possible relationships between swine and human influenza as human epidemics rise and fall In the same spirit of international cooperation and joint solution of problems of animal human disease is Blood s paper on The Pan American Zoonoses Center

In conclusion at the risk of being redundant may I say that far and above the valuable technical contributions to our understanding of animal disease and human health there must have come to the reader as it has to me an appreciation of the vastness of the interrelationships between animal and human disease and of a compelling need for a concerted integrated multidisciplinary team approach to their problems which can no longer due to our growing awareness be separated into distinct categories of animal as opposed to human disease No one discipline can provide the technical prowess necessary for the solution of problems of such complex ecology this has been made abundantly clear throughout this publication Furthermore no one discipline can afford the luxury of neglecting to seek the aid of others Animal and human health problems reside within the same continuum they cannot be removed and studied as isolates Their mutually inclusive interdependence thrusts itself upon us daily It is my firm conviction that if we took the energies that we expend in duplicating inquiries and studies and all too often in exalting one discipline while disparaging another and converted a portion of these energies to the organization of a horizontal partnership we should much more rapidly fill in the missing gaps in our knowledge of disease and more quickly solve the problems that face us This I am sure is what J D Martin meant by the sharing of problems as well as knowledge This then I hope to leave with you in summary

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